QUADRUPLE THERAPY FOR THE MANAGEMENT OF TREATING *HELICOBACTER PYLORI* INFECTION WITH HERBAL AND CONVENTIONAL MEDICINES

Ph. D Thesis

*By*

HAFIZ MUHAMMAD ASIF
(B.E.M.S, M. Phil)

PROF. DR. USMAN GHANI KHAN, Research Supervisor
PROF. DR. NAVEED AKHTAR, Co, Research Supervisor

*Department of Basic Medical Sciences*
*Faculty of Eastern Medicine*
*HAMDARD UNIVERSITY*
*Karachi - 74600*
*2012*
QUADRUPLE THERAPY FOR THE MANAGEMENT OF TREATING *HELICOBACTER PYLORI* INFECTION WITH HERBAL AND CONVENTIONAL MEDICINES

This thesis is submitted in partial fulfillment of the requirement for the degree of

Doctor of Philosophy (Eastern Medicine)

By

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PROF. DR. NAVEED AKHTAR, Co, Research Supervisor

Department of Basic Medical Sciences
Faculty of Eastern Medicine
HAMDARD UNIVERSITY
Karachi - 74600
2012
DEDICATED

TO

MY TEACHER

PROF. DR. USMAN GHANI KHAN

AND

MY BELOVED PARENTS

MUHAMMAD EESA AND MUSSARAT BEGUM
CANDIDATE DECLARATION

I, Hafiz Muhammad Asif hereby declare that the research work in this thesis entitled “QUADRUPLE THERAPY FOR THE MANAGEMENT OF TREATING HELICOBACTER PYLORI INFECTION WITH HERBAL AND CONVENTIONAL MEDICINES” carried out in the Faculty of Eastern Medicine, Hamdard University Karachi, Pakistan under the supervision of Prof. Dr. Usman Ghani Khan. This is my own original research work and no part of this thesis has been previously submitted for any degree of any University of Pakistan and abroad.

Hafiz Muhammad Asif

June 15th, 2012

Pakistan
Certificate

I, Prof. Dr. Usman Ghani Khan hereby certify that the research work in this thesis entitled “QUADRUPLE THERAPY FOR THE MANAGEMENT OF TREATING HELICOBACTER PYLORI INFECTION WITH HERBAL AND CONVENTIONAL MEDICINES” is the original research work carried out under my supervision in the Faculty of Eastern Medicine, Hamdard University Karachi, Pakistan. I certify that no part of this thesis has been previously submitted for any degree of any University of Pakistan and abroad.

Prof. Dr. Usman Ghani Khan

June 15th, 2012

Pakistan
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SUMMARY

Introduction

*Helicobacter pylori* is a small, gram-negative microaerophilic bacterium that regularly colonize, inhabit and persist in the mucus layer of the human stomach. More than 20 species of *Helicobacter* has been recognized. It causes a chronic low-level inflammation of the stomach lining and is strongly linked to the development of duodenal and gastric ulcers and stomach cancer. More than 50% of the world's population harbor *Helicobacter pylori* in their upper gastrointestinal tract. Infection is more prevalent in developing countries, and incidence is decreasing in developed countries. Emerging antibiotic resistance has consequences a major problem for the efficacy of treatment. Hence, research in *Helicobacter pylori* epidemiology and its associated diseases is therefore important for the development of novel treatment strategies and prevention.

Materials and methods

A study was conducted to evaluate the efficacy of Pylorex plus, a herbal formulation for the treatment of *Helicobacter pylori* infection as compared to Quadruple allopathic therapy (Omeprazol, Amoxicillin, Metronidazol and Bismuth compounds). The therapeutic evaluations of these medicines were conducted on 176 clinically and immunologically diagnosed cases of *Helicobacter pylori* infection. All the patients selected for the study were thoroughly examined and clinical history was recorded in the prescribed proforma of case sheet enclosed herewith the thesis. The therapeutic evaluation of the drug was made on the basic improvement in the subjective signs and symptoms, clinical observations and pathological investigations at periodic intervals during the course of treatment. This data was collected in the period April 2010-March 2012 and completed the clinical trials.
**Objectives**

To investigate the safety and efficacy of Pylorex plus, Herbal coded formulation (Test group) in comparison with Quadruple allopathic therapy (Control group).

**Methods**

One seventy six *H. pylori* positive patients (males:97, females:79, mean age: 36±12 year, range: 18-55) were enrolled in the study and divided into two groups according to treatment regimens. Quadruple therapy (Omeprazole; 20mg capsule, Amoxicillin; 500mg, Metronidazole; 500mg and bismuth compound; 400mg) was prescribed for 7 days and alternate phytomedicine-based quadruple formulation (Pylorex plus 500 mg tablet contains *Curcuma longa* rhizomes; 150 mg, *Mallotus philippenensis* fruits; 150 mg, *Glycyrrhiza glabra* roots; 100 mg and *Zingiber officinale* rhizomes; 100 mg) was prescribed for 15 days. C¹³-urea breath and stool antigen (HpSAg) tests were performed at baseline and after 1 month of treatment. The details of relevant gastrointestinal symptoms (abdominal pain, regurgitation, heart burning, indigestion and flatulence, nausea, vomiting and belching) were filled for each patient, using a special scoring system (absent: 0, mild: 1, moderate: 2, severe: 3).

**Outcome measures**

- Primary efficacy parameter: Clinical response.
- Secondary efficacy parameter: Laboratory investigation.

**Results**

*Helicobacter pylori eradication status*

According to the statistical analysis *Helicobacter pylori* was eradicated in 51 patients (56.66%) out of 90 patients by the use of Quadruple allopathic therapy (Control drug) and in 53 patients (61.62%) out of 86 patients by the use of Pylorex plus (Test drug). Comparison of data
recorded by participants relating to these variables showed no significant differences between test and control groups \((p>0.05)\). Chi-Square Test was applied and \(p\)-value was calculated as 0.3031 which is greater than 0.05 indicating that Pylorex plus and Quadruple therapy is equally significant in *Helicobacter pylori* eradication.

**Improvement in *H. pylori* associated symptoms**

There was a significant improvement in *Helicobacter pylori* associated symptoms in test group as compared to control group when observed between these two treated groups at the end of therapy. We recorded the intensity of symptoms as absent: 0, mild: 1, moderate: 2 and sever: 3 at baseline \((T0)\), 2\(^{nd}\) week of treatment \((T2)\) and after 4 weeks \((T4)\) of treatment through median values, interquartile ranges (IQR) and Wilcoxon signed-rank test was applied to calculate differences in median values. In test group a statistically significant decrease in the overall dyspeptic symptom score was observed from baseline \((T0:\text{median 8, IQR 6-10})\) to 2\(^{nd}\) week \((T2:\text{median 3, IQR 2-6})\) and one month after treatment \((T4:\text{median 3.5, IQR 3-7})\). Quadruple therapy also exhibited a statistically significant decrease in the overall dyspeptic symptom score from baseline \((T0:\text{median 9, IQR 7-11})\) to 2\(^{nd}\) week \((T2:\text{median 4, IQR 3-5})\) and one month after treatment \((T4:\text{median 6, IQR 3-7})\). In non *H. pylori* eradicated patients a marked symptomatic improvement was observed in test group in overall symptom score from baseline \((T0:\text{median 9, IQR 5-12})\) to one month after treatment \((T4:\text{median 4, IQR 2-6})\) as compared to quadruple therapy \((T0:\text{median 9, IQR 5-13})\) to one month after treatment \((T4:\text{median 8, IQR 5-10})\).

**Conclusion**

The findings from this randomized clinical trial revealed that there was no statistically significant difference when comparing the effectiveness of herbal medicine Pylorex plus (Test) to quadruple allopathic therapy (Control) for the treatment of *H. pylori* infection. Furthermore, it
is clearly evident that Pylorex plus possesses a therapeutic value in the improvement of \textit{H. pylori} associated symptoms as compared to Quadruple allopathic therapy. However, further clinical trials on larger scale and studies pertaining to mechanism of Pylorex plus are required before prescribing it as an alternate eradication therapy against \textit{H. pylori}. In summary, this study outlines an approach to the scientific and clinical validation of traditional and conventional medicines, so in its ultimate dictate; this is worthwhile exercise, since it leads to new class of therapeutics.

There was no untoward manifestation associated with the use of Pylorex plus and this has found good acceptability by all treated patients. The principal objective on herbal medicine Pylorex plus as compared to Quadruple allopathic therapy is to determine whether these may represent a platform for the development of novel therapeutic. This is an exercise of applying modern techniques and clinical design to product that have been in use for centuries.
Acknowledgement

I remain in gratitude to Almighty ALLAH for His mercy and kindness upon me to execute the clinical research described in this dissertation.

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HAFIZ MUHAMMAD ASIF
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<tr>
<td>μm</td>
<td>Micrometer</td>
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<tr>
<td>PCR</td>
<td>Polymerase Chain Reaction</td>
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<td>DNA</td>
<td>Deoxyribo Nucleic Acid</td>
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<td>flaA</td>
<td>Flagellin A</td>
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<tr>
<td>flaB</td>
<td>Flagellin B</td>
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<td>LPS</td>
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<td>cagA</td>
<td>Cytotoxic Associated gene A</td>
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<td>vacA</td>
<td>Vacuolating cytotoxin A gene</td>
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<td>Interferon-γ</td>
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<td>sIgA</td>
<td>Secretory Immunoglobulin A</td>
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<td>NUD</td>
<td>Non Ulcer Dyspepsia</td>
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<td>MALT</td>
<td>Mucosa Associated Lymphoid Tissue</td>
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<td>GERD</td>
<td>Gastroesophageal Reflux Disease</td>
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<td>UBT</td>
<td>Urea Breath Test</td>
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<tr>
<td>PPI</td>
<td>Proton Pump Inhibitor</td>
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<td>Adrenocorticotropic Hormone</td>
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<td>Interquartile Ranges</td>
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<td>IARC</td>
<td>International Agency for Research on Cancer</td>
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<td>MPM</td>
<td><em>Mallotus phillipes</em> (Lam.) Muell.</td>
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<td>Vanilloid Receptor Type 1</td>
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<td>HpSAg</td>
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Publications


5) Muhammad Akram, E. Mohiuddin, **H. M. Asif**, Khan Usmanghani, Helicobacter Pylori, Kansas Journal of Medicine, pp 119-123, 2011


CHAPTER -1

INTRODUCTION
1. Introduction

Bacteria were discovered in the human stomach for more than a century ago. In 1923 a spiral rod like bacterium was first discovered in the human stomach by Konjetzny. However, diseases of the upper gastrointestinal tract due to an infectious agent were documented by Warren and Marshall in 1980s when they isolated Helicobacter pylori (H. pylori) from gastric biopsies and an association was established between bacterium and gastritis and peptic ulceration. After this discovery, many diagnostic tests and antibiotic treatment strategies for H. pylori infection have been developed. However, despite this massive research, H. pylori colonisation is still highly prevalent in developing countries. Emerging antibiotic resistance has consequences a major problem for the efficacy of treatment. Hence, research in H. pylori epidemiology and its associated diseases is therefore important for the development of novel treatment strategies and prevention [1, 2, 3].

1.1 Microbiology

H. pylori is microaerophilic gram-negative rods that regularly colonize, inhabit and persist in the mucosal layer of stomach. More than twenty species of Helicobacter has been recognized in which many species still require formal recognition. Microaerophilic characteristic is unique in all the species of Helicobacter and most of the species are oxidase and catalase positive and some species have been identified as urease positive. Initially were described as Campylobacter pyloridis Campylobacter pylori having the cells usually curved or spiral shaped between 0.5 and 5 μm in length, with 5 – 7 sheathed flagella which are unipolar and 3 μm in length approximately. These flagella allow rapid motility through the viscous mucus layer of the human stomach. H. pylori produces an enzyme urease that converts host urea into ammonia and
carbon dioxide. *H. pylori* is well supported to survive uniquely in the human stomach due to its urease production, flagella motility and microaerophilia characteristics [4, 5, 6].

![Helicobacter pylori](http://www.google.com.pk/search?tbm=isch&hl=en&source=hp&biw=1014&bih=423&q=h.pylori&gbv=2&oq=h.pylori&aq=f&aqi=g6g)

**Figure 1: Helicobacter pylori**

(1.2) **Genome**

*H. pylori* is genetically heterogeneous and genomes are sequenced approximately 1.7 Mbp in size and have 35-40% content of a G+C. It consists of rRNA genes with two copies of the 16S, 23S and 5S. Its heterogeneous characteristics are considered to be happened mainly due to rearrangement of DNA and the foreign sequences may be introduced or deleted which results in every infected individual carrying a distinct strain genetic heterogeneity [7, 8].

(1.3) **History**

*H. pylori* was discovered first time in the human stomachs in 1982 by Dr. Barry Marshall and Dr. Robin Warren of Perth, Western Australia. They isolate it in the patients suffering from gastric mucosal inflammation and ulceration [9]. It was believed at the time that it is impossible
for the bacterium to survive alive in the human stomach due to the production of large amount of acid in the human stomach. Both scientists were awarded Nobel Prize in 2005 in Physiology or Medicine in recognition of their discovery and rewriting the textbooks on what causes gastric inflammation and ulceration in human stomach. In 1875 spiral-shaped bacteria were discovered in mucosal layer of human stomach by German scientists, but their results were not documented because they were unable to culture it. In 1893 same shaped bacteria were identified by Italian researcher Giulio Bizzozero in the stomach of dogs. In 1899 sediments of human gastric mucosal washings were studied in Jagiellonian University in Kraków by Professor Walery Jaworski. During his investigations, he found some spiral rod shape bacteria, which he named as *Vibrio rugula*. Then a possible role of this organism in the production of gastric diseases was established by this researcher. These investigations were published in the *Handbook of Gastric Diseases*. In the early 1900s several small studies demonstrated the pathogenesis of gastritis, stomach cancer and peptic ulcers due to the presence of curved rods in the stomach [10, 11, 12, 13, 14].

In 1970s bacteria were visualized in the stomach of patients suffering from gastric inflammation and ulceration and role of bacteria was established in the development of gastrointestinal diseases. In 1979s, Australian pathologist Robin Warren also observed the bacterium. He carried out further investigations on it in 1981 with Australian physician Barry Marshall. Both remain unsuccessful to culture the bacteria from the human stomach in many attempts but finally in 1982 they achieved their goal to visualize different colonies of bacterium when their Petri dishes unintentionally left in laboratory for 5 days on incubation over the Easter weekend. Then, Marshall drank a beaker of *H. pylori* culture for the purpose of observations. After few days he suffered from gastrointestinal symptoms such as stomach pain, heart burning,
nausea and vomiting. Signs of gastric inflammatory disorder were determined and the presence of *H. pylori* was confirmed in endoscopy after ten days. These findings revealed that *H. pylori* was the causative factor for inflammation of stomach. They also demonstrated that antibiotics are choice of treatment in gastritis. They described in their original publication that infection by this bacterium might cause stomach inflammation and ulceration which was thought to be happen by tension and stress or overtaking of spicy foods previously [15,16].

In 1987 triple therapy was suggested for the treatment of duodenal ulcers by Sydney gastroenterologist Thomas Borody. In 1994, an opinion was documented that *H. pylori* is the causative agent for the recurrent duodenal and gastric ulcers from National Institutes of Health (USA) and antibiotics are recommended in the treatment regimen [17].

### 1.4 Epidemiology

Geographical variations have been reported in the prevalence of *H. pylori* infection. More than 70-80% of the population in some developing countries is *H. pylori*, even many studies shows some documented reports about the prevalence of *H. pylori* infection at young ages. Prevalence of *H. pylori* in some developed countries usually has been documented fewer than 40% and is considerably high in adults and elderly people than in children and adolescents. Epidemiology and prevalence of *H. pylori* has a link with socioeconomic status in some geographical distribution particularly a close relation has been documented during childhood. In Western countries, the prevalence of *H. pylori* is higher among the first and second generation immigrants from the developing areas of the world. Prevalence of *H. pylori* infection remains relatively constant in developing countries. In the industrialized world its prevalence is rapidly decreasing. Improved hygiene and sanitation environment has resulted in this reduction of prevalence in early childhood. The rate of infection in developing countries rapidly rises in the
early life period and remains constantly high after acquisition of infection. But the prevalence of *H. pylori* infection in developed world is low in childhood life and slowly increases with age. In the Western world, the acquisition of new *H. pylori* infections at later stage is not more than 0.5% annually; which reflects a birth cohort effect with higher prevalence of *H. pylori* infection among the elders. Active elimination of *H. pylori* infection is acquired due to good hygiene and sanitary environment in children. So it can be concluded that children are usually at high risk to this new infection and lasts for long period of life unless treated specifically [18,19,20].

### 1.5 Prevalence of *H. pylori* infection in Pakistan

The prevalence of *H. pylori* infection in Pakistani population is well documented in different studies. In a report from Nilore, Islamabad, prevalence of *H. pylori* infection is 66.5% in dyspeptic population which is considered very high suggesting that *H. pylori* is the main etiological factor of dyspepsia than high acid production. A study was carried out by H. Qureshi and W. Ahmad on *H. pylori* eradication concluding that the infection rate is about 83% in adult patients undergoing upper GI endoscopy for dyspeptic symptoms [19]. Prevalence of *H. pylori* in Lahore had found 43.6% in dyspeptic population in a study by Mohsin *et al.* [20], whereas 21-60% prevalence in Karachi recorded by Shahana *et al.* [21]. There is some variation in prevalence rates within country which may be due to water and sanitary conditions and the intrinsic properties of diagnostic methods and sampling techniques used by researchers and investigators.

An overall prevalence of *H. pylori* in Pakistani dyspeptic patients have been recorded 84.6% in *Helicobacter* genus-specific PCR in which 66 out of 78 biopsy samples were positive. *H. pylori* was isolated in 53% of duodenal ulcer cases in some early prevalence reports from Pakistan and 80% of individuals having symptoms of upper gastrointestinal diseases were found
to be *H. pylori* positive [22]. In a report from Yakoob *et al.*, 2004 56% infection rate was documented in which PCR was used to amplify 16S rRNA gene in patients presenting with various gastric symptoms [22]. Several other studies by Proenca Modena *et al.*, 2007; Soylu & Ozturk, 2008 have revealed that there is a strong association between gastrointestinal disorders and the presence of *H. pylori* in the gastric mucosa [23].

### 1.6 Global prevalence

*H. pylori* has been isolated from different parts of the world in dyspeptic population in a quiet different ratio, highly prevalent in developing countries as compared to industrialized countries. Incidence of *H. pylori* has been reported in the range of 31-78% from different areas of the world including Japan, Brazil, Canada, Korea and Turkey [24]. The prevalence of *H. pylori* determined by endoscopy among British immigrant symptomatic Indian community was 52% as compared to 43% in white population [25]. Different factors are involved for the difference in prevalence between ethnic groups or races e.g., hygiene conditions, environmental contamination, water contamination, standards of living and socioeconomic conditions. Level of income and education is inversely associated with *H. pylori* infection [25].

### 1.7 Transmission and Sources of Infection

The exact rout of transmission of *H. pylori* is still doubtful. Usually *H. pylori* has been isolated from human stomachs and in some nonhuman primates as well. Pet animals are also determined as host for *H. pylori* thus, the presence of pets may be a source of infection and risk factor for *H. pylori* transmission. Zoonotic transmission of this bacterium is not documented in any case. Direct human-to-human transmission is the source for new infections and it may be either an oral-oral or faecal-oral route or both. The bacterium, *H. pylori* has been isolated in saliva, vomitus, gastric refluxate, and faeces of human, but it is stated that there is some
confusion for predominant transmission through any of these sources. It is revealed that gastroenterologists, dentists, nurses and partners of an \( H.\ pylori \) positive spouse have not been documented at high risk of \( H.\ pylori \) carrier. So it can be stated as a conclusion from all previous studies that \( H.\ pylori \) infection mostly occurs in early ages of life and close family members may be the source of infection. Prevalence of this bacterium is highly associated with childhood crowding and poor hygienic conditions in and outside the family, whereas crowding it is not so much associated among adults. Naked DNA or dead bacterium has been reported in environmental water sources in many studies. \( H.\ pylori \) has been isolated and cultured from waste water and faecal contaminated water sources has been done. Outbreaks of gastroenteritis among institutionalized young people supported the spread of infection via faecal contaminants. \( H.\ pylori \) also survive briefly on refrigerated food, so possible sources may also include contaminated foods [26].

### 1.8 Pathogenesis and virulence factors of \( H.\ pylori \)

\( H.\ pylori \) are extracellular, gram-negative rods, having flagella, and motile are the earliest major characteristics. A complex cascade of interactive mechanism develops between bacteria and host in the pathogenicity and virulence of \( H.\ pylori \). It has been investigated that many bacterial factors are present which play a vital role for colonization of bacterium in human gastric mucosal cells, e.g. proteins flagellin that support in the active transportation of the \( H.\ pylori \) to the surface of gastric mucosa which is encoded on \( flaA \) and \( flaB \) genes. \( H.\ pylori \) produces a transient hypochlorhydria after the infusion in the stomach mucosa. Interactions occur between glycolipids of cell-surface and \( H.\ pylori \) for adhesion. Lipopolysaccharides (LPS) are also present in its cell wall which induces mucosal integrity disruption. These lipopolysaccharide (LPS) are composed of a core oligosaccharide, a lipid moiety (lipid A) and an
O-chain polysaccharide which are thought to contribute to immune evasion [27, 28].

Urease enzyme is secreted by *H. pylori* which is essential for its survival that allows *H. pylori* to maintain a constant periplasmic and internal pH, which is essential for trans-membrane potential difference. The level of urease activity differs significantly between different *H. pylori* strains. Urease causes the mucosal damage by producing ammonia which is thought to be cytotoxic to epithelial cells. Overall a complex cascade of events starts after the attachment of *H. pylori* to gastric mucosa that causes injury of the tissues, in a same manner like all gram negative bacteria react [28]. *H. pylori* alters normal gastric secretion, increased serum level of gastrin in patients with duodenal ulcer due to *H. pylori* which results in increases production of acid. *H. pylori* also releases some proteins those are pathogenic to mucosa and causes cell injury. Cytotoxic-associated gene (*cagA*) produces a CagA protein which has immunogenic properties and associated with clinical presentation of the infection.

All *H. pylori* infected patients are presented with varying level of gastritis, but if the infection persists for long time and not treated properly then it may leads to severe gastric inflammation, atrophic gastritis, peptic ulceration and gastric adenocarcinomas also developed in *cagA* positive strains. Furthermore, vacuolating cytotoxin A gene (*vacA*) produces a protein which is also induces mucosal injury. An inflammatory reaction is stimulated by *H. pylori* that results in the clinical features of active infection such as neutrophilic gastritis. Phagocytic cells, T and B lymphocytes are stimulated. Inflammatory mediators or lymphokines such as interleukins (IL) 1, 2, 6, 8, 12, tumor necrosis factor–α (TNF) and interferon gamma are released by host. Rate of apoptosis (mucosal programmed cell death) is also an additional pathogenic manifestation of *H. pylori* infection [29,30,31,32].
1.9 Host immunological response to *H. pylori*

Two types of host immune responses against bacterial infections have been identified i.e. an innate response and an adaptive response. The first response is usually an initial and non-specific response towards bacterial infection, which reacts quickly with the aim of killing the bacteria. Adaptive immune response is antigen-specific and delayed process that stimulates the process of activation of T and B lymphocytes and memory cells [33].

1.9.1 Innate immunity

Toll-like receptors (TLRs) can identify the bacterial molecules in the innate immune response which are present on monocytes and dendritic cells (DCs) usually considered as antigen-presenting cells (APCs). Proinflammatory cytokines such as IL (interleukin)-1β and IL-8, TNF-α (tumour necrosis factor-α) are released after the bacterial contact with monocytes and
other APCs. Increased production of these inflammatory mediators or cytokines in *H. pylori* infection stimulates chemotaxis and granulocytic infiltration. Innate immune response against *H. pylori* infection is especially due to TLR in epithelial cells. But the gastric mucosal cell lines do not response to *H. pylori* LPS if in a small concentration but a high concentration of this endotoxin stimulate the immune process [34,35].

It has been discussed in several studies that a rapid stimulation of necrosis factor-κB and interleukin-8 expression takes place after an interaction between *H. pylori* and gastric mucosal cells. *H. pylori* has the ability to stimulate the NF-κB and IL-8 due to presence of some antigenic protein on its surface. Furthermore mitogen-activated protein kinases (MAPKs) have also been identified with stimulation of NF-κB, as mediators of *H. pylori* induced IL-8 expression. Interleukin-8 gene expression in *H. pylori* infection is always dependent upon activation and stimulation of both NF-κB and AP-1 (via activation of MAPKs) which shows that synergistic interactions between AP-1 and NF-κB are needed for maximal *H. pylori*-induced IL-8 production [36].

1.9.2 Adaptive immunity

**Cellular response**

Gastric inflammation is caused in virtually all *H. pylori* infected peoples. *H. pylori* infection also stimulates adaptive immune responses. Initially in the inflammatory response, neutrophils are stimulated, followed by stimulation of macrophages, T- and B lymphocytes, plasma cells, as well as injury and degeneration of some epithelial cells.
Host response is initially started after the invasion and attachment of \textit{H. pylori} to the gastric mucosal epithelial cells. Then a number of antigenic substances, including urease, LPS and HSP (heat-shock protein), all of which are involved in the activation of macrophages and T-cells. Epithelial tight junctions are disrupted that enhances antigen presentation to the lamina propia and induces more immune stimulation. Increased production of inflammatory mediators or cytokines such as IL-1, IL-6, TNF-\(\alpha\) and IL-8 are the actual responding agent. Furthermore, increased production of CD4/CD8 T-cell ratio causes chronic active gastritis within the gastric epithelial cells. Th1-predominant immune response in \textit{H. pylori} infection is also identified which is characterized by the induction of IFN-\(\gamma\) (interferon-\(\gamma\)) and IFN-\(\gamma\)-related genes. \textit{H. pylori} infection and severity of gastritis is associated with mucosal expression of the TNF-\(\alpha\) subunit CD68 and IFN-\(\gamma\) [37,38].
Inflammatory and immune response are induced by the host genetic characteristics in *H. pylori* infection. A strong cytokine pro-inflammatory mediator IL-1β encoded by the *IL-1B* gene is a powerful inhibitor of gastric acid secretion and plays an important role in *H. pylori* infection to initiate and stimulate the inflammatory response [39].

**Humoral response**

Strong specific systemic and local antibody response to the infection in individuals inhabited with *H. pylori* has been discussed in many studies. Complement system is activated due to *H. pylori* strains even in the absence of specific antibodies either via the classical pathway or alternative pathway. Primarily an active mucosal antibody IgA isotype is stimulated in *H. pylori*-infected individuals investigated in gastric secretions. In healthy individuals sIgA (secretory IgA) response is consistent. Anti *H. pylori* antibodies (sIgA) are also present and identified in salivary secretions and breast milk. Monoclonal antibodies are produced in *H. pylori* infections which cross react with gastric epithelium studied both in mice and humans. Furthermore, it has been discovered that induction of these antibodies alone sufficient to produce gastric inflammation in mice. Clyne et al have reported that a bactericidal effect is exerted on *H. pylori* on human serum taken from both infected and non-infected individuals. Moreover serum samples of the organism showed heat inactivation the killing effect on the organism which strongly suggests that it was complement mediated. Clyne et al. observed in a study that serum samples of the serum taken from infected subjects killed the *H. pylori* more effectively than serum collected from non-infected individuals, concluding that some of this effect is stimulated and induced by the classical pathway [40,41,42].
1.10 Clinical manifestations of *H. pylori* associated diseases

*H. pylori* is considered as uniquely inhabited to the mucosa of human stomach. Characteristics of different infecting strain, the environmental factors and the host factors probably lead to a range of subclinical and clinical outcomes. Inhabitation of *H. pylori* in the stomach mucosa does not stimulate any inflammatory or disease process in the host but some factors may enhance the chances of production and stimulation of various clinical manifestations in the upper gastrointestinal tract. Achlorhydria is usually seen in the first few months after infection that may lead to other gastrointestinal illness. Colonization is largely quiescent and persists for decades after this period. Diagnosis of *H. pylori* through different testing techniques is required to find out the etiological factors of gastrointestinal disorder such as gastritis, peptic ulceration, and gastric mucosal carcinoma. In these gastrointestinal inflammatory conditions, a positive *H. pylori* test further reveals a proper management of this bacterium i.e., to eradication treatment should be started immediately. But a negative *H. pylori* test indicates the further investigations to find out the other causative agents and proper preventive measures. Hence, there is a great need to understand the interaction between *H. pylori* and gastroenterological disorders and better knowledge is required to eradicate this bacterium to prevent these disorders [43,44,45].

1.11 *H. pylori* associated disease

Histologicl changes are usually initiated in all *H. pylori* infected individuals but clinical signs appear only in minority. *H. pylori* infection usually found to be associated with non-ulcer dyspepsia (NUD), acute and chronic gastric inflammation, gastric and duodenal ulceration, gastric carcinomas, lymphoma of mucosa associated lymphoid tissue (MALT) in stomach, non-Hodgkin’s lymphoma and in some cases iron deficiency anaemia and coronary heart diseases are
associated. It is estimated that chances of mucosal ulceration in *H. pylori* positive patients is 10 to 20% and distal gastric cancer may develop in 1 to 2% patients. Development and progression of these disorders mainly depends on the host and environmental factors. Severity and pattern of gastritis depends on both these factors in which *H. pylori* infection [46].

1.11.1 Gastritis and gastric lymphomas

*H. pylori* inhabitation always induces the production and invasion of neutrophilic and mononuclear cells in the gastric mucosa particularly in antrum and corpus of stomach. This is a primary condition resulting after *H. pylori* colonization. Superficial chronic gastritis and then atrophic gastritis are resultant *H. pylori* associated disorders. Gastric carcinoma appears in the cascade of these cellular changes. Gastric mucosa associated lymphoid tissue (MALT) lymphomas and adenocarcinoma of the antrum and body of the stomach is mostly associated in *H. pylori* infection if not treated properly. These lymphomas are mainly due to lymphocytic infiltration of the mucosal stroma inducing cellular alteration and proliferation. Chances of gastric carcinoma are high in patients with severe multifocal atrophic gastritis. A research study conducted on rates indicating a 90-fold increase when compared with normal controls. DNA is damage by different cytokines and free radicals as a result of chronic inflammation leading to carcinogenesis. Some antigenic proteins are also produced by *H. pylori* those stimulate the production of lymphocytes in the early stages of development of neoplasia [47].

It is investigated in a recent study that nonspecific symptoms of dyspepsia may results in acute phase of *H. pylori* infection such as nausea/vomiting, fullness of stomach, indigestion and pangastritis. Hypochlorhydria is indicated in this phase often lasting for months. The level of acid secretion disturbs if colonization persists for long period of time having a correlation with the distribution of gastritis. Destruction and loss of parietal cells may leads to reduction in acid
secretion which usually happens in atrophic gastritis, but it can be observed when acid secretory properties are normal but functional inhibition of parietal cells as recorded in vagotomy and over use of those drugs which leads to suppression of acid production in the stomach e.g., proton pump inhibitors (PPIs) [48,49].

![Image of H. pylori induced gastritis](http://www.google.com.pk/imgres?q=h.pylori+pathogenesis&num=10&hl=en&gbv=2&biw=1014&bih=423&tbnid=t_XViUQ9kRS2VM:&imgrefurl)

**Figure 4: H. pylori induced gastritis**

1.11.2 Peptic ulcer disease

*H. pylori* infection is strongly associated to gastric and duodenal ulcer diseases. Areas which are mostly exposed to gastric acids such as lesser curvature of the stomach and duodenal bulbs are the commonest site for the development of ulceration. Some host and bacterial factors are involved in the development of ulceration. Sever mucosal inflammatory sites are the predominant ulcers sites. Pyloric and duodenal ulcer disease results when the production of acid is normal to high, initiating severe inflammatory response in the distal stomach and proximal
duodenum. Ulceration of these areas may leads to intestinal complications such as mucosal perforation with bleeding and stricture formation. Bleeding is recorded in 15 to 20% of the infected persons and is considered as most common and severe complication of ulcer disease [50]. Ulceration in the duodenal areas is more common as compared to gastric ulceration in Western countries; whereas in other regions prevalence of gastric ulcers is more common. Gastric ulcers are usually reported at the ages of above 40 years while duodenal ulceration mostly investigated between 20 and 50 years of age. Approximately 85% of gastric ulcers and 95% of duodenal ulcers investigated in *H. pylori* infected individuals in the early periods. Prevalence of ulcer disease and its recurrence can be reduced by *H. pylori* eradication therapy in early periods of infection [51].

In Western countries, the incidence and prevalence of peptic ulcers diseases has gradually reduced during the last two decades. In some recent studies, annual incidence is two to three cases of peptic ulceration per thousands *H. pylori* positive cases. This reduction is due to some associated factors such as early use of eradication therapy, safe hygiene and sanitary environment and low family members. In this geographical distribution the proper diagnosis and treatment of *H. pylori* in ulcer patients has decrease the casual role of *H. pylori* in peptic ulcer diseases [52].

**1.11.3 Non-ulcer Dyspepsia**

Non-ulcer dyspepsia may also be associated with *H. pylori* infection which comprises reflux, dysmotility and ulcer like symptoms. Non-ulcer dyspepsia may be due to some other possible factors including lifestyle factors, stress, changes in the secretion of gastric acids and emptying and increased serotonin sensitivity. Psychosocial impairment e.g., depression, somatization and anxiety are also included in the etiological factors of non-ulcer dyspepsia.
Persons suffering from these psychosocial impairments are found to be twice in non-ulcer dyspepsia as compared to be positive for *H. pylori*. Therefore, therapies for eradication of *H. pylori* usually does not resulting in the improvement of symptoms related to non-ulcer dyspepsia. So only *H. pylori* eradication therapy cannot be considered the standard treatment in all the patients suffering from non-ulcer dyspepsia [53,54].

### 1.11.4 Gastroesophageal Reflux Disease (GERD)

Gastroesophageal reflux disease is represented with different clinical manifestations e.g., esophagitis, Barrett’s esophagus and a possible relationship exist between these disorders and *H. pylori*. It is investigated that gastroesophageal reflux disease may occur independently of *H. pylori* infection. It is investigated in some studies that *H. pylori* inhabitation can decreases the risk for developing esophagitis and Barrett’s esophagus. This inverse protective effect is thought to be due to the acid suppressive effect of *H. pylori* induced corpus predominant gastritis. This concept of inverse protection has been supported in many prevalence studies. Barrett’s esophagus can be protected in CagA positive strain. Incidence of esophagitis increases after eradication of the organism, investigated by Labenz and his colleagues. In some cases symptoms of GERD are exacerbated if *H. pylori* eradication therapy is given to the patients. Endoscopic findings are not strongly associated in GERD and similarly pH probe measurements and histology examination are not promoted in GERD. Consequently, more studies are required to find out a relationship between *H. pylori* infection and gastroesophageal reflux disease [55,56].
1.12 *H. pylori* infection and associated factors

*H. pylori* infections can lead to the development of gastritis in almost all individuals but all cases do not develop gastric cancer. Some host genetics factors, inflammatory response from individuals, environmental factors such as diet, hygienic conditions smoking and bacterial strain virulence are also important in determining the disease outcome [57]. Levels of expression of IL-1 and other cytokines against *H. pylori* infection may be affected by host genetic polymorphisms. Greater risk individuals with pro-inflammatory genotypes have of corpus predominant pangastritis which further leads to atrophic gastritis and gastric carcinomas. Environmental factors, such as diet, hygienic conditions and tobacco consumption play an important and key role in further investigations of the outcome of *H. pylori* associated disorders. Prevalence of
peptic ulcer disease is also increased due to smoking. Increased risks of developing gastric cancer in individuals whose diets are high in red meat and salt. But, it has been suggested that a diet rich in fruit, vegetables and cereals have mild to moderate prophylactic potential for gastric cancer, however the specific food constituents having protective effects for gastric cancer still remain to be completely defined [58].

1.13 Classification of symptoms of H. pylori infection

H. pylori related symptoms can be divided in to two categories;

Group 1 (classic H. pylori symptoms)

Group 2 (non-classic H. pylori symptoms)

Major symptoms that are associated to H. pylori infection are labeled as classic symptoms and Group 1. Group two contains symptoms that H. pylori causes in a less obvious manner. The details of both group is given in Table 1. Some serious health conditions and H. pylori associated disorders are also given below in the table [59, 60].
### Table 1: *H. pylori* related symptoms and complication

<table>
<thead>
<tr>
<th>Group one (classic)</th>
<th>Group two (Non classic)</th>
<th>Developed/Serious Health Conditions</th>
<th>Complications or associated diseases</th>
</tr>
</thead>
<tbody>
<tr>
<td>Abdominal pain</td>
<td>Anxiety</td>
<td>Autoimmune diseases</td>
<td>Gastritis</td>
</tr>
<tr>
<td>Heart burning/Acid reflux</td>
<td>Depression</td>
<td>Heart problems</td>
<td>Peptic ulcer disease</td>
</tr>
<tr>
<td>Regurgitation</td>
<td>Fatigue</td>
<td>Cancer</td>
<td>Non peptic ulcer dyspepsia</td>
</tr>
<tr>
<td>Nausea/vomiting</td>
<td>Headache</td>
<td>Osteoporosis</td>
<td>GERD</td>
</tr>
<tr>
<td>Constipation</td>
<td>Sinus problem</td>
<td>Ulcers</td>
<td>Malt lymphoma</td>
</tr>
<tr>
<td>Diarrhea</td>
<td>Weight gain</td>
<td></td>
<td>Stomach Cancer</td>
</tr>
<tr>
<td>Bad Breath</td>
<td>Weight loss</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Chest pain, pain between shoulder blades</td>
<td>Sleep disorders</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Skin problems:</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Urticaria. Rosacea, Hives, etc</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

1.13. **Group 1 (classic *H. pylori* symptoms)**

1.13.1 **Pain abdomen and acid reflux**

*H. pylori* can cause pain in different ways. *H. pylori* causes inflammation in the mucosa of stomach and small intestine that ultimately leads to pain. Moreover, *H. pylori* can also disturb
the digestive process, constipation, diarrhea and gases may be produced in the gut which may leads to sensation of pain. Moreover, if \( H. \text{ pylori} \) is present in the stomach for a long time, it damages the parietal cells that produce hydrochloric acid that can literally burn the delicate lining of the stomach causing pain. The acid can also spill back up the esophagus causing reflux. This leads to low stomach acid, or hypochloridria. When acid is low, it becomes very difficult to digest food. As a result, food sits in the stomach and putrefies, giving off gases and causing a burning sensation in the stomach and/or throat. In the latter situation, anti-acid medication will make the problem worse and will, in fact, make it easier for the \( H. \text{ pylori} \) to survive. Studies have shown that despite the fact that \( H \text{ pylori} \) is well adapted to live in the acidic conditions of the stomach, the bacteria will migrate and live in areas of the stomach where the environment is less acidic \[59, 60\].

1.13.2 Anemia

Many studies have shown that iron deficiency anemia is closely linked with \( H. \text{ pylori} \) because \( H. \text{ pylori} \) infection reduces production of stomach acid; it becomes difficult to digest food, particularly protein. If food cannot be digested in the stomach, the absorption of minerals can be affected. Iron is the only mineral to have been studies extensively but over time it is likely that multiple mineral and vitamin deficiencies develop as a result of \( H. \text{ pylori} \) infection \[61.62\].

1.13.3 Bloating & excessive gas

Inflammation in the digestive system can cause bloating and the production of gases as digestion of food becomes more compromised. Undigested sugars and fats in the intestines may leads to overgrowth of bacteria and yeasts. As the bacteria and yeast feed on the undigested food, gases are given off, leading to abdominal distension and flatulence \[63\].
1.13.4 Chest pain & Pain between the shoulder blades

Inflamed in the stomach due to *H. pylori* infection causes pain reflexes into adjacent areas of the body because stomach is innervated by nerves from the 4th-8th thoracic vertebrae. These vertebrae correspond to stomach areas as well as chest and shoulder blades. Severe chest pain may be produced due to *H. pylori* infection [64].

1.13.5 Constipation

Food is not properly digested in the stomach in *H. pylori* infection because *H. pylori* causes low stomach acid by damaging the parietal cells of the stomach. As a result domino effect may be produced due to this undigested food when it is released into the intestine creating ‘backs-up’ the entire digestive system [65].

1.13.6 Diarrhea

*H. pylori* can contribute to diarrheal symptoms resulting due to infections and inflammation in the intestinal areas. Once the body rids itself of the unwanted organisms or toxins, the intestines usually return to normal. The symptoms may become chronic if the infection is chronic in nature, as happen in most *H. pylori* cases. The diarrhea may not be constant and may happen infrequently, or it may happen most days. Intestinal damage caused by *H. pylori* may lead to intestinal weeping (similar to the way burns and wounds to the skin weep). This can also contribute to diarrhea and may result not only from *H. pylori* but also from food sensitivity. If sugars and fat are not absorbed properly because *H. pylori* is preventing proper digestion in the stomach and intestines, fluid is drawn into the colon. If the colon cannot reabsorb this fluid, the stool will become loose and watery. Bacteria and yeasts can also feast on these undigested food particles, causing gas, flatulence, cramping and bloating [65].
1.13.7 Nausea & Vomiting

It is not clear understood that how *H. pylori* causes nausea and vomiting. It can be assumed that body tries to rid itself of the infection by ejecting it through the vomiting process. Nausea and vomiting are common symptoms of *H. pylori* and may be confused for pregnancy morning sickness in women [65].

1.13.B *H. pylori* Symptoms: Group Two (non-classic symptoms)

1.13.8 Anxiety

Any time there is a *H. pylori* problem or any digestive inflammation (from foods, other digestive infections); the adrenal glands have to produce the stress hormone cortisol. It is common to see either too high or too low levels of cortisol in people with *H. pylori*. Each molecule of cortisol has to be made from a molecule of progesterone and over time this leads to a deficiency in progesterone. In women, this often causes mood problems, especially depression, irritability and anxiety. It can also lead to PMS symptoms such as painful menstruation, heavy bleeding or skipping periods altogether. As the adrenal glands make cortisol, other hormones such as DHEA, testosterone and estrogen can also drop too low, again contributing to depression in men and women [66].

1.13.9 Fatigue / Low Energy

Inadequate intake of food due to loss of appetite leading to chronic digestive infections also lead to a condition called adrenal fatigue. Adrenal glands have to produce the stress hormone cortisol in digestive inflammation. If the adrenals become tired, energy levels tend to become depleted, especially in the mid-afternoon [67].
1.13.10 Migraines

Studies have shown that migraines are cured when *H. pylori* has been eradicated but its mechanism is still not very clear. It may be due to immune responses, hormone imbalances and neural factors caused by *H. pylori* contributing to the development of headaches. For example, digestive infections can cause low progesterone in women and progesterone deficiency can cause headaches, particularly during the second half of the menstrual cycle. In addition, food sensitivities, possibly triggered by *H. pylori* may also contribute to headaches and migraines [68].

1.13.11 Sinus problems

Inflammatory problems in the stomach may affect the mouth, lungs or even the eyes and ears. *H. pylori* is closely associated with yeast and fungal overgrowth and research clearly indicates that sinus problems are often nothing more than fungal problems [69].

1.13.12 Skin: Urticaria. Rosacea Hives, etc.

It is not clear how *H. pylori* cause skin conditions. Research and clinical experience has shown that these conditions can improve significantly when *H. pylori* is removed from the body and relevant dietary changes are made [70].

1.13.13 Sleep problems

Melatonin is a hormone that helps in sleep process. If there is a *H. pylori* problem or any digestive inflammation, the adrenal glands have to produce the stress hormone cortisol. It is common to see either too high or too low levels of cortisol in people with *H. pylori* infection. High cortisol can disrupt the body’s ability to make melatonin, which can cause insomnia. Each molecule of cortisol has to be made from a molecule of progesterone and over time this leads to a deficiency in progesterone. In women, this can lead to sleep problems [71].
1.13.14 Weight gain

When *H. pylori* causes a stress response where the adrenal glands release hormone cortisol. One of the effects of high cortisol is to encourage body fat storage round the middle of the body (stomach, spare tyre). Adrenals that are overworking can also slow down the thyroid gland. It is well known that slow or sluggish thyroid function can lead to weight gain. As the adrenals are called upon to make more and more cortisol, they start to fatigue and cortisol begins to drop. Low cortisol leads to a situation where fats cannot be metabolized and used by the body and as a result they are stored away in the fat cells. At the same time, many women and even men, especially middle-aged men, become estrogen dominant. In women, estrogen is dominant over progesterone and in men it becomes dominant over testosterone. This leads to weight gain and also muscle loss [72].

1.13.15 Weight loss

*H. pylori* infection leads to a stress response where cortisol levels become elevated. Cortisol causes the body’s lean tissues i.e., muscle and bone to be broken down for emergency fuel. As these tissues break down, bodyweight can start to drop. *H. pylori* infection may also result in poor digestion of food. If the building blocks of the body amino acids from protein and fatty acids from fats and oils cannot be absorbed into the body, it will continue to break down as the body becomes more and more malnourished [72].

1.14 Developed/Serious Health Conditions

1.14.1 *H. pylori* and autoimmune conditions

An autoimmune condition is characterized by the immune system attacking its own tissues. Example include multiple sclerosis, thyroiditis, colitis, crohn’s disease, type I diabetes and fibromyalgia. *H. pylori* has been linked with all these diseases but the links are quite tenuous
at this stage. It is believed that the proteins on the surface of *H. pylori* are very similar to the proteins found in the body’s own tissues. The immune system may confuse these proteins and instead of attacking *H. pylori*, mount an attack against its own tissues instead. The research seems to indicate that the thyroid gland is the most likely target of this autoimmune complication [73].

1.14.2 *H. pylori* and heart disease

There is growing scientific evidence to support the role of various infections including *H. pylori* in the development of heart disease. The precise mechanisms are not yet known, but it seems as though the problem is related to the way in which our immune systems recognize certain proteins in our heart and blood vessels and confuse them for proteins found on the surface of *H. pylori*. This is known as ‘molecular mimicry’. Studies have shown associations between *H. pylori* infection and homocysteine, cholesterol, blood pressure and insulin resistance. These are potential risk factors for heart disease and may also be part of the mechanism by which *H. pylori* could cause heart disease [73].

1.14.3 *H. pylori* and osteoporosis

*H. pylori* eventually leads to a condition called ‘hypochloridia’, or low stomach acid. When stomach acid is low, the breaking apart of proteins and release of minerals like calcium and magnesium is compromised. If the body cannot digest food and absorb nutrients properly, calcium and magnesium levels may drop. Of course, calcium and magnesium are essential for bone health and deficiencies can lead to a reduction of bone density. *H. pylori* cause chronic inflammation in the stomach and intestine, which causes an elevation in the hormone cortisol from the adrenal glands. High levels of cortisol actually cause bone to be broken down. In addition, when cortisol is too high, progesterone becomes too low. Progesterone is needed to
build bone. In short, *H. pylori* can lead to osteoporosis because it indirectly leads to a lack of minerals in the body that are the building blocks for bone, it increases bone turnover through high cortisol and reduces bone building through low progesterone (this is why women are more at risk of osteoporosis than are men) [74].

1.15 Diagnostic criteria for *H. pylori* infection

An individual can be identified as *H. pylori* positive by different modern diagnostic techniques. There are some limitations, advantages and disadvantages of each technique according to the condition and severity of the patients. The diagnostic tests depend upon whether endoscopic biopsy is necessary or not. Endoscopic biopsy is usually performed to obtain the histologic evaluation for further culture and examination. Polymerase chain reaction (PCR) and rapid urease tests are also performed with endoscopic biopsy. Serology, urea breath tests (UBT), and stool assays are usually preferred which are non invasive diagnostic techniques. *H. pylori* can be isolated in salivary secretions, feces, and dental plaque by PCR amplification. Brief descriptions of these diagnostic procedures are given below [75,76].

1.15.1 Histological evaluation

*H. pylori* infection can be diagnosed through histologic testing and this method was previously considered the gold standard diagnostic technique for the confirmation of active infection. This method requires the endoscopy to obtain a tissue. This is advantageous because it is a definitive diagnosis of infection, severity of inflammation. It is also considered as confirmatory test to identify the presence or absence of gastric carcinomas and MALT lymphoma but inadequate numbers of biopsy specimens are obtained in many cases or failure to obtain proper specimens from different parts of the stomach has proven its disadvantages and limitations. In many cases, staining techniques are also required, which is disadvantageous due to
its high costs and longer processing times [77, 78].

1.15.2 Culture

Culture has an important role in studies of growth factors and metabolism as well as antibiotic susceptibility studies. Culture techniques are limited in diagnosis of *H. pylori* infection because it is difficult to grow on culture media. It is also has some limitations because costly, time-consuming, and high labor involvement is required to perform this test. Therefore this method is not considered in routine investigation as primary test for the confirmation and diagnosis of *H. pylori* infection [78].

1.15.3 Polymerase Chain Reaction (PCR)

Polymerase chain reaction is being effective for evaluation and identifying the *H. pylori* in easily sampled tissues such as salivary secretion and dental plaques. *H. pylori* infection can be diagnosed and classified with the advent of this modern diagnostic technique. Epidemiologic studies and pathogenic evaluation can be done to identify different strains of bacteria. *H. pylori* can be identified and isolated in small samples through PCR and this method requires no especial transport and processing. This method has the advantage of being performed rapidly with cost effectiveness. But currently limited laboratories have this facility to perform PCR. Moreover, false-positive results can occur because in previously treated patients because the segments of *H. pylori* DNA can be detected in PCR in the gastric mucosa. Some false-negative results due to some human errors may also occur while interpretation of bands on electrophoretic gels included in its limitations and disadvantages [79].

1.15.4 Rapid Urease Testing

Urease enzyme is produces by *H. pylori* which converts urea into ammonia and carbon dioxide. Samples are obtained from *H. pylori* infected persons through endoscopy and placed in a
medium which contain urea. Urea will be converted in to carbon dioxide and ammonia in the presence of urease. Due to this reaction pH of the medium will be increased and pH-dependent indicator indicates a subsequent color change. This test is advantageous because it is commonly available, less expensive and fast. But urease activity can be decreased if the person has the history of recent use of antibiotic agents, acid suppressing agents such as proton pump inhibitors and bismuth salts. These factors may leads to false-positive results indicating the limitations of this test [80].

1.15.5 Urea Breath Test

Urea breath test is a modern technique and considered as gold standard test now days for diagnosis of *H. pylori* infection. This method is also used after the completion of eradication therapy to observe the effects of treatment. Active infection of *H. pylori* can be determined by urease activity. Patients are advised to ingest either $^{14}$C or $^{13}$C urea which will be converted in to ammonia and carbon dioxide in the presence of urease. Carbon dioxide is then absorbed and expired out in the breath, where it can be detected easily confirming the presence of active infection or eradication of organism after treatment. $^{13}$C urea is considered as safer due to nonradioactive properties and hence it is advantageous in children and women of childbearing age. Moreover urea breath test is advantageous because it does not require endoscopy, relatively inexpensive and easy to perform. But it is of limited value if the patient has recently given eradication therapy such as antibiotic agents, proton pump inhibitors and bismuth compounds. Therefore, there should be a discontinuing of antisecretory medications at least 1 week before testing for active infection and for confirmation of eradication [81].

1.15.6 Serologic Tests

Serologic tests are very advantageous to identify the organism because these methods are
easy, fast and relatively less expensive. IgG antibodies to *H. pylori* are present in infected persons and can be isolated by use of a biochemical assay. The immune system typically responds through the production of immunoglobulins (Ig), which are specific to the antigens of the organism. These antibodies can be confirmed easily in serum analysis or samples of whole blood. Certain strains of more virulent *H. pylori* can be identified by serologic techniques by detecting antibodies to virulence factors. These virulence factors are associated with more severe disease manifestation such as gastritis, ulceration, gastric carcinomas and lymphoma. However, eradication of *H. pylori* cannot be confirmed by this method because some different samples at different occasion of time and titer changes in specified amounts are required. False-positive results and a low positive predictive values are also expected therefore this test should be a second-line methodology [82,83].

**1.15.7 Stool antigen test**

Enzyme immunoassay techniques are also preferred to identify presence of the organism such as stool antigen testing is a non invasive method that can determine the *H. pylori* antigen in stool samples. Enzyme immunoassay has a better sensitivity and specificity as compared to other non invasive tests. It is considered as advantageous due to its low cost and fast results. It is also a reliable procedure to identify active infection as well as to confirm the eradication after successful treatment [84].

A comparative analysis of advantages and disadvantages is given in Table 2.
Table 2: Diagnostic testing for *H. pylori*

<table>
<thead>
<tr>
<th>Endoscopic Testing</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Histology</strong></td>
<td>Excellent sensitivity and specificity</td>
<td>Expensive and requires infrastructure and trained personnel</td>
</tr>
<tr>
<td><strong>Rapid urease testing</strong></td>
<td>Inexpensive and provides rapid results. Excellent specificity and very good</td>
<td>Sensitivity significantly reduced in the post treatment setting</td>
</tr>
<tr>
<td></td>
<td>sensitivity in properly selected patients</td>
<td></td>
</tr>
<tr>
<td><strong>Culture</strong></td>
<td>Excellent specificity, Allows determination of antibiotic sensitivities</td>
<td>Expensive, difficult to perform, and not widely available. Only marginal sensitivity</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Non Endoscopic Testing</th>
<th>Advantages</th>
<th>Disadvantages</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Polymerase chain reaction</strong></td>
<td>Excellent sensitivity and specificity. Allows determination of antibiotic sensitivities</td>
<td>Methodology not standardized across laboratories and not widely available</td>
</tr>
<tr>
<td><strong>Antibody testing</strong> (quantitative and qualitative)</td>
<td>Inexpensive, widely available, very good NPV</td>
<td>PPV dependent upon background <em>H. pylori</em> prevalence. Not recommended after <em>H. pylori</em> Therapy</td>
</tr>
<tr>
<td><strong>Urea breath tests (13C and 14 C)</strong></td>
<td>Identifies active <em>H. pylori</em> infection. Excellent PPV and NPV regardless of <em>H. pylori</em> prevalence. Useful before and after <em>H. pylori</em> therapy</td>
<td>Reimbursement and availability remain inconsistent</td>
</tr>
<tr>
<td><strong>Fecal antigen test</strong></td>
<td>Identifies active <em>H. pylori</em> infection. Excellent positive and negative</td>
<td>Polyclonal test less well validated than the UBT in the post treatment setting. Monoclonal test appears reliable before and after antibiotic therapy. Unpleasantness associated with collecting stool</td>
</tr>
<tr>
<td></td>
<td>predictive values regardless of <em>H. pylori</em> prevalence. Useful before and after <em>H. pylori</em> therapy</td>
<td></td>
</tr>
</tbody>
</table>

PPI = proton pump inhibitor; PPV = positive predictive value; NPV = negative predictive value; UBT = urea breath test.
1.16 General diagnostic guidelines

There are some difficulties while observing the patients suffering from *H. pylori* infection which includes which patients should be advised to go in laboratory and which test should be recommended at what time. These problems usually depend on the patient’s conditions such as patient economical status, patient’s preference of invasive or non invasive test, availability of different diagnostic methods at patient’s locality. Positive and negative predictive value of different methods is also an important element which depends on the prevalence of *H. pylori* population in a specific region. Invasive methods such as endoscopy and biopsy should be preferred only in those cases that need a confirmation of some serious condition like gastric carcinoma, MALT etc besides *H. pylori* infection. Testing is based on the symptoms of associated disorders like gastritis and peptic ulcer diseases. Relieve in the symptoms of simple dyspepsia is not conclusive evidence for the eradication of the infection in patients, and to advice any test without previous history of symptoms and ulceration is not recommended advice. Urea breath test (UBT) and stool antigen tests are the gold standard diagnostic methods. Both tests are non invasive and most reliable and cost effective procedures to confirm the active infection status. Serologic evaluation is also better technique to confirm if there is a previous history of gastritis or ulceration. A better confirmation can be achieved by endoscopy and biopsy in cases with a history of peptic ulcer diseases, as well as confirmation about the gastritis and gastric cancer etc. Follow-up testing should be done and eradication must be confirmed by stool antigen or urea breath tests because specificities and sensitivities of these methods are more than 90%. These confirmatory tests should be recommended after four weeks of completion of eradication therapy [85, 86].
1.17 Management

1.17.1 General treatment guidelines

*H. pylori* lives in an environment which is very difficult to access in many therapies and emerging microbial resistance has produced many difficulties in its optimum treatment. Furthermore, it is very difficult for patients to take many of the recommended regimens because there are some problems with compliance of the drugs such as to ingest many tablets at least twice thrice times daily with some adverse effects as well. However, current therapies are obtaining the cure rates more than 85% in most populations [87].

1.17.2 Vaccination

*H. pylori* is prevalent all over the world and is responsible for significant mortality and morbidity. Eradication of *H. pylori* is going to be very difficult and expensive; therefore vaccine therapy is necessary for its prevention. In early 1990s vaccination to prevent this infection was invented based on the models of murine. Production of cytokines or interleukins (IL) 4 and 10 stimulate helper T cells phenotype 2 which is the basic mechanism of protective immunity against the organism instead of antibody development against organism [88].

There are several issues remain in consideration for the development of a safe and effective vaccine to prevent this infection. A safe mucosal host adjuvant to stimulate an immune response must be defined in the development of vaccines. Route of administration should be defined particularly. Different studies on mice indicated that nasal and rectal routes are safe which do not leads to gastritis after immunization that usually results in oral route. Specific *H. pylori* antigens e.g., urease with *Escherichia coli* Cholera toxins are used as conjunctive agent with varying level of success and toxicities. Attenuated live vaccines, including *H. pylori* antigens and strains of *Salmonella*, are used in combination. Moreover, different agents are
needed to be developed for the purpose of complete sterilization of the gastric mucosa [89].

1.17.3 Antibiotic drugs

Now days, antibiotics are commonly prescribed to eradicate \textit{H. pylori} infection. Antibiotics are recommended in combination with some other agents because monotherapy have no solid results due to low efficacy and development of resistance. Activity of Metronidazole is independent of pH of the stomach, but resistance to this drug has been identified. However this resistance can be limited to some extent, when it is used with clarithromycin. Clarithromycin has 7	extendash}11\% resistance rates but is not suitable in acid environment and can leads to dysgeusia. Moreover, it is expensive as compared to other antibiotic drugs. Resistance with Amoxicillin is very low but its activity is pH-dependent and not prescribed as a single remedy. It is usually given in combination with some adjuvant drugs such as proton pump inhibitors (PPI). Tetracycline resistance is also low and also has the advantage of low cost but photosensitivity reactions and discoloration of the teeth is disadvantageous [90,91].

1.17.4 Adjunctive agents

Some adjunctive agents are prescribed in combination with antibiotic agents. The most popular adjunctive agents are the proton pump inhibitor which is currently used to eradicate \textit{H. pylori} infection in combination with omeprazole. Omeprazole acts by inhibiting microsomal enzymes of bacteria and also increases the intragastric pH, thereby enhancing the action of antibiotic. It also helps in increasing antibiotic concentrations and reducing gastric secretions in the stomach. Some other adjunctive agents are also used such as ranitidine bismuth citrate and histamine receptor antagonists. Bacterial cell wall is interrupted with bismuth compounds helping to eradicate the bacterium [92].
1.17.5 Current therapies

Nowadays the most popular and effective regimens includes triple therapy which is the combination of 2 antibiotic agents and 1 adjunctive agent (Triple therapy) at least for 14 days. It is documented in a previous report that adequate eradication rates have been achieved with 7 days quadruple therapy that includes 2 antibiotics, 2 adjunctive agents. Now most physicians are recommending triple drug therapy or quadruple drug therapy to eradicate *H. pylori* infection. American College of Gastroenterology recommended the guidelines in 1998 for the eradication of *H. pylori* as follows:

A) Triple therapy which includes 2 antibiotic agents i.e., clarithromycin and either metronidazole or amoxicillin and 1 adjuvant agent; proton pump inhibitor at least for 14 days

B) Triple therapy which includes 2 antibiotic agents; amoxicillin or tetracycline clarithromycin and 1 adjuvant agent; ranitidine bismuth citrate prescribed for 14 days

C) Quadruple therapy which includes metronidazole and tetracycline with proton pump inhibitor and bismuth compounds for 14 days

Treatment of *H. pylori* should be started with triple therapy and if it is failed on follow-ups, then further rescue therapies should be given including a different combination of antibiotic drugs with increased the duration of treatment. Quadruple therapy may also be started on failure of triple therapy [93].

The decoding of the complete genome of *H. pylori* has indicated many convincing remedies and new combinations chemotherapeutic world. It is now possible to formulate new active agents that act on particular vital protein products essential for the survival of the bacterium. Some new compounds have been developed due to current emerging drug resistance of *H. pylori* against antibiotics due to which its eradication is going to be very difficult.
Nitazoxanide is used with omeprazole as an effective agent. Furthermore, macrolides other than clarithromycin may act as convincing drug in the future [94].

1.17.6 Side effects

Antibiotic treatment of *H. pylori* infection is not without risk. Antibiotic therapy can lead to the development of pseudomembranous colitis, a potentially severe infection caused by *Clostridium difficile*. In addition, antibiotics frequently enable the overgrowth of Candida albicans, which can result in vaginitis, gastrointestinal disturbances, or other complaints. Moreover, antibiotic treatment could lead to the overgrowth of antibiotic strains of *H. pylori*, making further attempts at eradication more difficult [95].
1.18 *H. pylori* and its associated disorders in Unani perspective

Unani system of medicine describes that the imbalance of humour (Akhat) in the body as the cause of disease. This imbalance leads to disturbance in the temperament of a person which is usually called as mal-temperament or so-e-mizaj. There are four akhat in Unani system of medicine: [96]

- Blood (Dam)
- Phlegum (Belgham)
- Bile (Safra)
- Black Bile (Sauda)

According to Unani system, every person has its own temperament which depends upon the quality and quantity of akhat. All four humours are present in a person but one humour may be dominant which demonstrate its temperament. These relevant imbalances are the root causes of a condition and must be balanced for real cure of disorders. In Unani system of medicine, diagnosis of disease is very important step in the management of any disease. During the process of diagnosing, clinical manifestation i.e. signs and symptoms are noted and then laboratory test are recommended. Temperament (Mizaj) is the basic diagnostic factor in this system. Quwwat-e-mudabbira-e-badan is thought to be a strong and basic power of body to combat any harmful environment and to maintain a normal equilibrium. If this power fails to maintain this condition then it may leads to qualitative and quantitative derangement in the akhlats (humors) of body. Akhlats (humors) are the factors which are important in the development of tissues and organs and maintain the function of organs. This abnormality or imbalance in akhat will cause
physiological and anatomical disturbance in the body leading to pathological changes in the form of disease [97].

Usoole ilaj i.e. principle of management is adopted after diagnosing the etiological factors of a disease. These are given as follows [98].

- Izalae sabab (removal of the cause)
- Tadeele akhlat (Balance of humors/akhlat)
- Tadeele aza (normalization of organs/system)

So, according to this theory inflammation (Warm) of stomach may be due to imbalance of these humors (Akhlat) and it is divided in two following types; [99, 100]

1. Inflammation due to Blood or Safra (Warm e damvi or warm e sfravi)
2. Inflammation due to Phlegham (Warm e belghami)
3. Inflammation due to Sauda (Warm e sodavi)
4. Inflammation due to Cancer (Warm e sartani)

### 1.18.1 Inflammation due to Blood or Safra (Warm e damvi or warm e sfravi)

This is due to the imbalance of blood or safra in the stomach. It is also called as hot inflammation. The signs and symptoms of this inflammation may be as fever, pain abdomen and heart burning. Inflammation can be felt at the site of stomach. Thirst and irritability will be very increased due to hotness in the stomach and loss of appetite is very common. Nausea and vomiting is very common in this type of inflammation [100].
1.18.2 Inflammation due to Phlegham (Warm e belghami)

This type of inflammation is due to the accumulation of excessive amount of phlegham in the stomach. This is loose inflammation resulting due to indigestive diet in the stomach that leads to production of belgham and weakness of stomach. It may be due to less exercise. The signs and symptoms of this inflammation will be opposite as compared to hot inflammation. There will be less fever and thirst. The colour of face and tongue will be white and body will be swollen [100].

1.18.3 Inflammation due to Sauda (Warm e sodavi)

Inflammation may be due to the disturbance in the suadawi khilt. There may be inflammation that will be tight. Symptoms of this warm may be tightness of stomach and pain. Irritability is very common and patient will be psychological. Body will be dry and rough and there will be deficiency of blood in body [100].

1.18.4. Inflammation due to Cancer (Warm e sartani)

This is a rare type of inflammation and it may be due to the production of any cancerous cell in the stomach. Sever pain and inflammation is felt at the site of stomach. Loss of appetite and irritability is very common. Patient loses his weight and deficiency of blood in the body is the major symptoms of this type of inflammation [100]. Some other external factors are also involved in the development of inflammation in the stomach enlisted below: [100]

✓ Overeating of hot and heavy diets.
✓ Overuse of chicken and spicy diet.
✓ Excessive use of raw vegetables.
✓ Indigestion.
✓ Accumulating of toxins in tissues that can block circulation.
✓ Malnutrition.
✓ Disturbance of the central nervous system.
✓ Physical and mental stress.
✓ Decrease in natural resistance and loss of immunity.

If this inflammation persists for long period of time then ulceration may result in the mucosa of stomach that is called Qaroh e maida. The symptoms become worse in ulceration particularly pain and bleeding is increased. Patient will feel pain in empty stomach due to acid secretion and this pain worsen after taking diet as well [100].

1.19 Unani treatment for H. pylori infection

Antibiotic resistant is going to be more severe drawback in the eradication therapy. Researches on alternative sources of antimicrobial agents are recommended over the last decade and experiments on the plant material are supported to achieve this goal. Traditional system of medicine comprises for more than hundreds of plants worldwide which are used for the eradication of bacterial agents. In vitro screening activity of these medicinal plants has been documented in many studies but clinical trials reports on these herbal medicines are lacking. Natural sources are usually safer than synthetic antibiotics and many physicians and patients prefer to use herbal medicines. Thus there should be a proper awareness to healthcare professionals about these documented herbal antibiotics.

In a recent study, some commonly used Unani medicine plants from Pakistan were screened to have anti-\textit{H. pylori} activity that are commonly used in gastrointestinal ailments to evaluate the natural agents for pilot compounds to eradicate \textit{H. pylori} [101].

In a recent study, investigators have evaluated that licorice extract produced a potent anti \textit{H. pylori} effects even those were resistant against clarithromycin. Researcher documented that
licorice extract may be the basic alternative source to eradicate *H. pylori* infection. In another study it is found that extracts of licorice are effective to treat *H. pylori* strains which were resistant to both clarithromycin and amoxicillin, providing a potent chemo-preventive source for gastric inflammation, ulceration and carcinomas [102].

Curcumin is the substance obtained from *Curcuma longa* contains many anti-inflammatory and powerful antioxidant compounds. It is considered as chemo-preventative agent and recently strong antibacterial effects have been documented against *H. pylori*. Studies have shown a significant in vitro effect of curcumin against *H. pylori*, indicating that *Curcuma longa* could be a potential source for the eradication of *H. pylori* infection [103].

*Zingiber officinale* is also a valuable traditional plant which is commonly prescribed for the treatment of many gastrointestinal problems such as dyspeptic problems, gastritis and indigestive disorders. Chemopreventative activity has also been reported in animal models. In vitro activity of ginger and its compounds have supported its uses to eradicate and treat *H. pylori* and its associated problems such as dyspepsia, development of gastric inflammation and ulceration [104].
CHAPTER -II

LITERATURE SEARCH
2. Literature Search

The coded herbal formulation *Pylorex Plus* for the treatment of *H. pylori* infection comprises of *Mallotus philippinensis*, *Curcuma longa*, *Glycyrrhiza glebra* and *Zingiber officinale*. The literature search of these plants supports their anti *H. pylori* activity as given herewith.

2.1 *Mallotus philippinensis* Muell.

<table>
<thead>
<tr>
<th>Botanical name</th>
<th><em>Mallotus philippinensis</em> Muell.</th>
</tr>
</thead>
<tbody>
<tr>
<td>English name</td>
<td>Monkey-face tree</td>
</tr>
<tr>
<td>Hindi name</td>
<td>Kamala, Kampillaka, Kapila, Shend</td>
</tr>
<tr>
<td>Synonyms</td>
<td>Kamala, Kameela</td>
</tr>
<tr>
<td>Family</td>
<td>Euphorbiaceae</td>
</tr>
</tbody>
</table>

![Figure 6, 7: Fruits and leaves of *Mallotus philippinensis*](http://www.google.com.pk/search?tbm=isch&hl=en&source=hp&biw=1014&bih=423&gbv=2&aq=+Mallotus+philippinensis&aq)

2.1.1 Description

Trees of *Mallotus philippinensis* are found to be used as source of medicine and located all over the tropical areas of India, Pakistan, Sri Lanka and North Western areas. The height of trees is about 10 meters and its flowers are dioecious. Leaves are alternate, articulated and rusty-
tomentose. Trigonous globular capsules are found commonly [105].

2.1.2 Chemical constituents

*Mallotus philippinensis* mainly comprise of 5,7-dihydroxy-8-methyl-6-prenylflavanone, 3′-prenylrubranine, red compound, isorottlerin, rottlerin. Resin 80 per cent, citric, oxalic and tannin acids, malotoxin, and paroxybenzoic acid [106].

2.1.3 Pharmacological action

Antibacterial, Anti-inflammatory, Antioxidant, Appetizer, Antitumor, Cooling, Purgative, Anthelmintic, Vulnerary, Detergent, Carminative [107,108].

2.1.4 Medicinal uses

Anti *H. pylori* activity of Kameela have been evaluated in many studies especially against clarithromycin resistant (CR) and metronidazole resistant (MR) strains. It could be hopefully utilized for the development of new antimicrobial agents to prevent *H. pylori* related disorders. It is mainly used in powder form which is obtained by crushing of the fruits or capsules of this herb. This powder is also used in other bacterial infections particularly in eye diseases. It is also a conventional remedy for the treatment of tape-worm. If it is taken internally, it removes leprous eruptions. The glands and hairs of the fruits are used to remove intestinal worms and also as a purgative [109].
2.2 *Curcuma longa* Linn.

**Botanical name**  
*Curcuma longa* Linn.

**English name**  
Turmeric

**Indian name**  
Haldi

**Family**  
Zingiberaceae

![Figure 8, 9: Rhizom of *Curcuma longa*](http://www.google.com.pk/search?tbm=isch&hl=en&source=hp&biw=1014&bih=423&q=curcumaklonga&gbv)

### 2.2.1 Description

*Curcuma longa* (Turmeric) is a rhizomatous herbaceous perennial plant belonging to the ginger family, Zingiberaceae. 20 °C and 30 °C temperature and a proper annual rainfall are needed to thrive it. Rhizomes are collected annually and then can be re-seeded in the relevant season. Curcumin is extracted after drying and powdering the root of the *Curcuma longa* plant by a solvent extraction [110].

### 2.2.2 Chemical constituents

Curcumin flavonoid (diferuloylmethane) is the major active constituents of turmeric and
various volatile oils, including zingiberone, tumerone and atlantone are also obtained. Other constituents are also present such as resins, sugars and proteins. Curcumin is the most important and potent active constituent which consists of 0.3-5.4 percent of raw turmeric [111, 112].

2.2.3 Pharmacological action


2.2.4 Medicinal uses

It is well known anti viral, anti fungal and anti bacterial plant, particularly inhibits *H. pylori*. *Curcuma longa* extract has significant anti-inflammatory effects and commonly prescribed in inflammatory disorders such as rheumatoid arthritis, gastritis and amyloid-beta (Alzheimer's polymers). It is also used in the treatment of carcinomas. Anti-inflammatory effects are due to inhibition of inflammatory pathway of Cox-2 but not Cox-2 itself. It has been reported as being used for ulcer treatment in some developed era. Anti cancer effects are due to apoptosis in various cancer cell types including stomach cancer, skin cancers etc [114, 115].
2.3 *Glycyrrhiza glabra* Linn.

**Botanical name**  
*Glycyrrhiza glabra* Linn.

**English name**  
Liquorice

**Synonyms**  
Lacrisse, sweet licorice, licorice root

**Family**  
Fabaceae

![Figure 10, 11: Roots and flower of Liquorice](http://www.google.com.pk/search?tbm=isch&hl=en&source=hp&biw=1014&bih=Glycyrrhiza+glabra+Linn)

2.3.1 **Description**

Liquorice is a perennial herb having height approximately 1m with 7–15 centimeters pinnate leaves and leaflets 9–17. Flowers are measured 0.8–1.2 cm long with purple to pale whitish blue colored. The fruit is 2–3 centimeters long with oblong pods containing many seeds. This plant is a legume that can be found in many areas of the world. It is not confused with Fennel or Anise which are also the sources of same flavoring agents [116].

2.3.2 **Chemical constituents**

It contains saponin and triterpenes which include glycyrrhetinic acid, glycyrrhizin and
liquiritic acid. Liquiritin, coumarins, asparagine, formononetin are major flavonoids and isoflavonoids. Sugars, polysaccharides and starch are also found in it [117].

2.3.3 Pharmacological action

It is found to be as anti-inflammatory, anti-ulcer, antibacterial, anti-viral, hepatoprotective, immune-stimulant, expectorant, spasmylytic, anti-catarrhal, laxative and demulcent [118].

2.3.4 Medicinal uses

Glycyrrhizin is a glycoside present in Liquorice which has similarities in structure and function as the adrenal steroids and is 50xs sweeter than sugar. Its functions are like adrenocorticotropic hormone (ACTH) causing retention of sodium and water and depletion of potassium. It has been reported in many studies showing good anti *H. pylori* activity against the resistant strains of clarithromycin and amoxicillin. It has been reported to have cortisone like anti-inflammatory activity and has been reported useful in inflammatory problems such as arthritis and allergic reactions. Liquorice has also been prescribed in hypoglycemia, Addison’s disease and other adrenal insufficiencies. In herbal medicines, it is considered as adaptogen and used in anti-cancer formula, which helps in regulation of the hypothalamic-pituitary-adrenal axis. It has been reported to be effective in autoimmune disorders including rheumatoid arthritis, lupus, scleroderma, and animal allergies. Liquorice used in traditional system of medicine for gastritis and peptic ulcer diseases. It also exhibits mild laxative action and commonly used as antiviral medication for ophthalmic, oral and genital herpes. Demulcent activity of liquorice has also been reported and used in bronchial complaints like coughs, flu and other complication of the pleural cavity [119, 120].
2.4  *Zingiber officinale* Linn.

**Botanical name**  *Zingiber officinale* Linn.

**English name** Ginger

**Common name** Adrak, Sonth

**Family** Zingibiraceae

![Figure 11, 12: Rhizomes of Ginger](http://www.google.com.pk/search?=Rhizomes+of+Ginger&pbx=1&oq=Rhizomes+of+Ginger&aq)

2.4.1  **Description**

*Zingiber officinale* or ginger is the plant containing rhizomes which is commonly used in medicine, delicacy and spices. It belongs to family Zingiberaceae. Some other common plants of this family are cardamom, turmeric and galangal [121].

2.4.2  **Active constituents**

Gingerols, shogaol, sesquiterpenoids with (-)-zingiberene. sesquiterpenoids (β-sesquiphellandrene, bisabolene and farnesene) in smaller amounts and monoterpenoid fraction (β-phelladrene, cineol, and citral) are the major compounds isolated from this plant[122].
2.4.3 Pharmacological action

Anti-inflammatory, antioxidant, anti tumor, digestive, antiemetic, stomachic, carminative, antibacterial [123, 124].

2.4.4 Medicinal uses

Ginger is commonly used as a source of medicine in Unani system of medicine to treat a wide range of ailments such as dyspepsia, peptic ulcer, motion sickness, and inflammatory diseases. It has been reported in vitro studies that growth of H. pylori can be inhibited by a standardized extract of ginger rhizome with a range of 0.78 to 12.5 μg/mL minimum inhibitory concentration. The extract was examined in a rodent model of H. pylori-induced disease. The extract was tested to Mongolian gerbils with daily doses of 100 mg/kg body weight in rations either three week before the infection or six weeks after the infection. Ginger extracts lower down the load of H. pylori when compared with controls. It also causes a reduction in acute and chronic inflammation, ulceration and epithelial cell degeneration caused by H. pylori [125, 126].

Cytokines are known to play a vital role in H. pylori-associated gastrointestinal disorders, and infection leads to a characteristic local inflammatory response in the gastric mucosa resulting in acute gastritis, which later leads to chronic gastritis. H. pylori and its enzymes can stimulate
neutrophils by directly stimulating these inflammatory cells or by stimulating the release of epithelial chemokines. *H. pylori* components such as LPS and proteins can attract and activate neutrophils and other inflammatory cells, hence stimulating the production of IL-1, 6, 8 and TNF-α. Extracts of ginger inhibits the activity of COX-2, the NF-κB transcriptional response, and the production of IL-1β, IL-6, and IL-8. Therefore, ginger extract may decrease *H. pylori*-induced acute and chronic inflammatory process through the inhibition of a number of components of this pro-inflammatory signaling pathway [127].
CHAPTER - III

AIMS AND OBJECTIVES
3. AIMS AND OBJECTIVES

Recent studies on prevalence of *H. pylori* infection have indicated that half of the world's population is suffering from this infection. Infection may leads to chronic gastric inflammation in all infected individuals. The management of *H. pylori* infection includes its proper diagnose, treatment and confirmation of eradication. Different treatment regimens to eradicate *H. pylori* infection have been investigated since its discovery in early 1990s. Now day’s antibiotics with some adjuvant agents are commonly prescribed to eradicate this infection but it is going to be failed due to antimicrobial resistance leading to treatments complications.

3.1 Aim of study

A multicenter clinical trial is designed to understand interaction of disease and its associated disorders, symptoms, context, patients response and the clinical skill vis a vis better management of *H. pylori* infection. This research study has a specific aim to investigate the impact of intensive medical intervention with herbal and allopathic medicine to treat *H. pylori* infection. During this study patients suffering from *H. pylori* infection were examined and given a treatment in different medical centers such as Shifa-ul-Mulk Memorial Hospital located at Hamdard University Karachi, Matab Hakeem N. Salik, Rawalpindi and Bahawalpur Victoria Hospital, Bahawalpur, Pakistan.

3.2 Objective

The main objective of this study is to prove the efficacy of herbal formulation as compared to allopathic medicine for patients suffering from *H. pylori* infection. In this context clinical investigation, diagnosis and treatment with test and control drugs was performed. The proposed test herbal medicine vis a vis control allopathic medicines have provided focal points for initiating, maintaining and to contribute to improved treatment and prevention of *H.
pylori infection. This trial is helpful to the development of evidence based herbal therapies for patients suffering from H. pylori infection.

3.3 Null hypothesis (Ho)

There is no significant clinical difference between the efficacy and safety of herbal verses allopathic medicine for the treatment of H. pylori infection.

3.4 Alternate hypothesis (H1)

Pylorex plus (Test) tablet is of greater value and will show great differences as compared to Quadruple therapy (control) for the treatment of H. pylori infection.

3.5 Alternate hypothesis (H2)

Quadruple allopathic (Control) therapy is of great value and will show great differences as compared to Pylorex plus (Test) for the treatment of H. pylori infection.

3.6 Statistical analysis

This is the set standard to decide the cut-off value between treatment groups when comparing the two groups. If the results are significant at this set level (α=0.05) the null hypothesis will be rejected.

3.7 Purpose

Bacterial infections including infection due to H. pylori can be treated with medicinal plants. Hundreds of plants are enlisted in traditional system of medicine which are commonly prescribed for these problems. But despite this broad use of herbal medicinal treatment there is no convincing evidence based data to evaluate the effectiveness and safety of these alternative therapies because these have no proper documented reports on controlled clinical trials. Traditional and herbal medicines have no proper patent rights which may be the one obvious reason for this fact. Furthermore herbal medicine has been found to be deficient to fulfill modern
methods for testing the efficacy and safety.

Therefore, systematic analysis of alternative treatment on *H. pylori* infection was evaluated and rigorous clinical investigation of Unani/Herbal medicine as compared to allopathic medicine was conducted. Specific objective for the current research undertaken on *H. pylori* infection was the performing research theme areas. Laboratory investigation, measuring epidemiological assessment, proper treatment and prevention of *H. pylori* infection as open comparative prospective was the main purpose of our study. Phase I trials of herbal test medicine intervention facilitated the design and conduct of randomized, control trial. The focus was on research methodology, biostatistics, clinical trial design and laboratory methods that relate to Unani and allopathic medicine for cure and prevention of *H. pylori* infection.

This research study was concluded after investigating the efficacy of herbal formulation as compared to allopathic medicine. Significance of the research on herbal medicine for the management of *H. pylori* infection is to address covering study design and procedures, sequence of clinical studies, translation of clinical data into statistical hypothesis, solution of outcome measure, safety and toxicity, inclusion and exclusion criteria and data analysis of the disease.
CHAPTER - IV

METHODOLOGY
4. METHODOLOGY

The study was based on an experimental clinical trial of herbal formulation Pylorex plus for *H. pylori* infection in which patients were randomly assigned to receive either herbal medicine or control allopathic treatment. Proper history and clinical examination were recorded on each follow up.

This is a case control, multicenter evaluation based study, conducted on the patients living near Shifa-ul-Mulk Memorial Hospital, Hamdard University, Karachi, Matab Hakeem N. Salik, Rawalpindi, Hakeem Muhammad Said Shaheed Memorial Research Center, Bahawalpur and Bahawalpur Victoria Hospital (BVH), Bahawalpur from April 2010-March 2012.

4.1 Diagnostic technique

Patients were examined clinically and having *H. pylori* related symptoms were enrolled in the study. A proforma was filled up before the start of treatment consisting of clinical features and investigations with other important data and was regularly filled up during the course of the treatment. Diagnosis of *H. pylori* infection was confirmed by stool antigen test which is the gold standard test to diagnosis *H. pylori* at baseline and one month after treatment. Some other laboratory investigations were also performed which includes, urea breath test, histological and culture evaluation and endoscopy in some cases.

4.2 Methods/Design

Study is randomized controlled trial in primary care with an open intervention. All patients examined by the General Physician and given either herbal or allopathic medicine for *H. pylori* infection. All patients were divided on the basis of treatment into two groups i.e., control group and test group.
4.3 The test group

The test group was presented herbal formulation Pylorex plus that comprises of different herbal medicinal plants components.

4.4 The control group

The control group was subjected to quadruple allopathic therapy

4.5 Eligibility

Ages eligible for study: 15 – 45 years

Genders eligible for study: Both male and female

Patients fulfilling inclusion and exclusion criteria which are mentioned below

Patients giving informed consent before treatment

4.6 Inclusion criteria

The cases were included in the study having the following criteria

1 The patients suffering from *H. pylori* infection

2 Patients having no previous record of treatment against *H. pylori* infection

3 Patients living in Karachi, Rawalpindi and Bahawalpur

4 Patients having no pathological complications on routine examination

5 All socioeconomic classes were included in the study

6 Male and female patients between 15 to 45 years of age

4.7 Exclusion criteria

The exclusion criteria for this trial were as follows:

1 Patients having surgical history of stomach or intestine were excluded
2 Patient with history of any previous herbal or allopathic medication were excluded

3 Patient with concurrent physical illness, for example uncontrolled hypertension and diabetes mellitus

4 Patient having history of adverse reaction to any of the study drugs as or contraindicated for their use

5 Pregnant females were also excluded due to safety measures

6 Patients suffering from complicated and serious conditions like coma, meningitis, and encephalitis or head injury

7 Patients hospitalized for any serious diseases

8 Patients with a previous history of drug interaction or abuse and those with known poor compliance were excluded from this trial

4.8 Patient’s withdrawal criteria

Patients were withdrawn due to following reasons;

- If the patient is not willing to continue
- Any acute systemic illness during the therapy
- Drug intolerance
- If the patient is not regular/not interested in therapy or compliance
- Severe adverse reaction or allergic reactions

All the patients gave verbal or written, informed consent for their participation, and the protocol was approved by the appropriate independent Ethical Committee in Faculty of Eastern Medicine, Hamdard University Karachi, Pakistan.
4.9 Independent variable

It includes the present and past history, personal history, family history and socioeconomic history, medical and surgical history.

4.10 Dependent variable

It includes abdominal pain, retrograde burning, regurgitation, indigestion and flatulence, anorexia, nausea, vomiting, general weakness, irritability, belching and hematemesis.

4.11 Confound variable

Age, sex, nutrition, pulse, blood pressure, temperature and respiration are confounding variables.

4.12 Sample size

Sample size estimated in clinical assessment on *H. pylori* infection has been carried out based on general physical examination, general appearance of the patients, age, sex, and local examination of the abdomen in a pilot study at Shifa ul Mulk Memorial Hospital. Trial was conducted on 176 patients suffering from *H. pylori* infection from both groups (90 patient from control and 86 from experimental group) between ages of 15-40 years irrespective of socioeconomic status.

4.13 Data collection

Clinical trial proforma was filled up to collect data through interviewing the patients, personal observation, and use of file and documents to maintain case records. The clinical trial proforma attached here which clearly specifies the clinical feature and information.

4.14 Statistical analysis

SPSS (Version 17) and Microsoft excels were used to for statistical analysis and p value was calculated by applying Chi Square test. All differences were considered statistically
significant if a ‘p-value’ calculated less than 0.05.

Likert scale was used to analyze the intensity of symptoms (scored as absent:0, mild:1, moderate:2, severe:3) such as abdominal pain, heart burning, regurgitation, indigestion and flatulence, nausea, vomiting, belching at baseline (T0), after 2 week (T2) and after 4 weeks of treatment (T4). Median values and interquartile ranges (IQR) were recorded to represent the level of improvement. The Wilcoxon Signed Ranks test was used before and after treatment to test a hypothesis about the intensity of symptoms by the location of median values.

4.15 Study limitations

Primary analysis was based on a urea breath test. The data was adjusted based on the number of cases in the light of demographic factor using statistical methods like multinomial logistic regression. The data were composed in separate group. The groups were compared after random selection of subject in equal proportion using SPSS software.

4.16 Ethical issues

Study was conducted under the rules of Ethical Committee (EC) of Shifa-Ul-Mulk Memorial Hospital, Faculty of Eastern Medicine, Hamdard University Karachi, Pakistan. Study design and protocols were presented to the board members of Ethical Committee (EC) and Board of Advance Studies and Research (BASR).

Ethical committee clearance and permission was obtained whenever necessary considering as under.

a) Informing each participant of the study and interviewing and examining the patient who consented to participate in the study.

b) Identity will not be revealed and the data would be kept strictly confidential.
c) Copy of the entire data will be made available to the Medical Superintendent Shifa ul Mulk Memorial Hospital.

d) The clinical trial protocols were approved by the committee. The test was performed when *H. pylori* infection was suspected particularly.

### 4.17 Dosage form design

#### 4.17.1 Control drugs

The allopathic quadruple therapy was selected for the purpose of comparison so as to obtain a reliable data. Doses were calculated according to patient’s condition and severity.

The details are given as follows:

#### 4.17.2 Quadruple allopathic therapy

Antibiotic agents in combination with adjuvant agents such as proton pump inhibitors and bismuth compounds being the most widely studied drugs to eradicate *H. pylori* infection. Bacterial microsomal enzymes are inhibited by Proton pump inhibitors (Omeprazole) as well as intragastric pH is also raised which facilitate the action of antibiotic agents by increasing antibiotic concentrations and reduction in gastric secretions [128].

##### 4.17.2.1 Omeprazole

**Generic name** Proton pump inhibitor

**Description**

Proton pump inhibitors (PPIs) are most potent inhibitors of acid secretion having a long-lasting reduction of gastric acid production. Proton pump inhibitors are superseded on H2-receptor antagonists who have different mode of action the similar effects. These groups are generally considered very effective and among the most widely selling drugs all over the world. The vast majority of these drugs are benzimidazole derivatives; however, promising new
research shows that imidazopyridine derivatives may be more valuable sources of treatment [129].

**Mechanism of action**

Hydrogen/potassium adenosine triphosphatase enzyme system (H+/K+ ATPase or gastric proton pump) of gastric parietal cell is blocked by proton pump inhibitors. The proton pump is the terminal stage in gastric acid secretion, being directly responsible for secreting H+ ions into the gastric lumen, making it an ideal target for inhibiting acid secretion. ("Irreversibility" here refers to the effect on a single copy of the enzyme; the effect on the overall human digestive system is reversible, as the enzymes are naturally destroyed and replaced with new species.) The proton pump inhibitors are given in an inactive form. The inactive form is neutrally charged (lipophilic) and readily crosses cell membranes into intracellular compartments (like the parietal cell canaliculus) that have acidic environments. In an acid environment, the inactive drug is protonated and rearranges into its active form. As described above, the active form will covalently and irreversibly bind to the gastric proton pump, deactivating it [130, 131].

**Pharmacokinetics**

In general, the absorption of proton pump inhibitors is unaffected by co-administration with food. Intake of concomitant food decreases the rate of omeprazole absorption. The absorption of lansoprazole and esomeprazole is decreased and delayed by food. It has been reported that these pharmacokinetic effects, however, have no significant impact on efficacy [132]. The elimination half-life of proton pump inhibitors ranges from 0.5–2 hours, however the effect of a single dose on acid secretion usually persists up to 2–3 days. This is because of accumulation of the drug in parietal cell canaliculi and the irreversible nature of proton pump inhibition [132].
Uses and indications

- Dyspepsia
- Peptic ulcer disease (PUD)
- Gastroesophageal reflux disease (GORD/GERD)
- Laryngopharyngeal reflux
- Barrett's esophagus
- Prevention of stress gastritis
- Gastrinomas and other conditions that cause hyper secretion of acid
- Zollinger-Ellison syndrome

Dose

The proton pump inhibitors are given 20mg twice daily for the period of 14 days before meal for the treatment of *H. pylori* infection [132].

Adverse effects and precautions

The most common adverse effects are headache, diarrhoea, abdominal pain, and nausea. Except for diarrhoea, the adverse effects of Proton pump inhibitor do not appear to be related to age, dosage, or duration of treatment. The diarrhoea seems to be related to the profound acid suppression, which has been shown to alter the bacterial content of the gut. Proton pump inhibitor is only contraindicated if the patient has a known history of hypersensitivity to them, and they should be used with caution in patients with severe hepatic disease. Omeprazole is a pregnancy category C agent; the others are pregnancy category B medications. Proton pump inhibitor is not recommended for use in breastfeeding mothers [133].

4.17.2.2 Amoxicillin

Generic name Amoxicillin
Brand name Amoxil, Dispermox, Trimox

Drug category

Amoxicillin belongs to a class of antibiotics called penicillin. Other members of this class include ampicillin (Unasyn), piperacillin (Pipracil), ticarcillin (Ticar) and several others [134].

Description

Amoxicillin (INN), formerly amoxycillin (BAN), amoxicillin (cilamox) in Australia, abbreviated Amox, Tormoxin (in India), is a moderate spectrum, bacteriolytic, β-lactam antibiotic used to treat bacterial infections caused by susceptible microorganisms. It is usually the drug of choice within the class because it is better absorbed, following oral administration, than other β-lactam antibiotics. Amoxicillin is susceptible to degradation by β-lactamase-producing bacteria, and so may be given with clavulanic acid to decrease its susceptibility [135].

Mode of action

These do not kill bacteria, but stop bacteria from multiplying by preventing bacteria from forming the walls that surround them. The walls are necessary to protect bacteria from their environment and to keep the contents of the bacterial cell together. Bacteria cannot survive without a cell wall. Amoxicillin is effective against many different bacteria including \textit{H. influenzae}, \textit{N. gonorrhoea}, \textit{E. coli}, \textit{Pneumococci}, \textit{Streptococci}, and certain strains of \textit{Staphylococci} [136].

Uses and indication

Amoxicillin is used to treat infections due to organisms that are susceptible to the effects of amoxicillin. Common infections that amoxicillin is used for include infections of the middle ear, tonsils, throat, larynx (laryngitis), bronchi (bronchitis), lungs (pneumonia), urinary tract, and skin. It also is used to treat gonorrhea [136].
Dose

For most infections in adults the dosing regimens for amoxicillin are 1 gm b.i.d, 250 mg every 8 hours, 500 mg every 8 hours, 500 mg every 12 hours or 875 mg every 12 hours, depending on the type and severity of infection [136].

Side effects

Side effects due to amoxicillin include diarrhea, dizziness, heartburn, insomnia, nausea, itching, vomiting, confusion, abdominal pain, easy bruising, bleeding, rash, and allergic reactions. Individuals who are allergic to antibiotics in the class of cephalosporin may also be sensitive to amoxicillin [136].

4.17.2.3 Metronidazole

Brand name Flagyl

Drug category Antibiotic, Amebicide and Antiprotozoal

Chemical name 2-(2-methyl-5-nitroimidazol-1-yl) ethanol

Description

Metronidazole is a nitroimidazole antibiotic medication used particularly for anaerobic bacteria and protozoa. Metronidazole is an antibiotic, amebicide, and antiprotozoal. It is the drug of choice for first episodes of mild-to-moderate Clostridium difficile infection [137].

Mechanism of action

Metronidazole, taken up by diffusion, is selectively absorbed by anaerobic bacteria and sensitive protozoa. Once taken up by anaerobes, it is non-enzymatically reduced by reacting with reduced ferredoxin, which is generated by pyruvate oxido-reductase. This reduction causes the production of toxic products to anaerobic cells, and allows for selective accumulation in anaerobes. The metronidazole metabolites are taken up into bacterial DNA, and form unstable
molecules. This function only occurs when metronidazole is partially reduced, and because this reduction usually happens only in anaerobic cells, it has relatively little effect upon human cells or aerobic bacteria [138].

**Uses and indication**

- Bacterial vaginosis, Pelvic inflammatory disease
- Anaerobic bacterial infections such as *Bacteroides fragilis, spp, Fusobacterium spp, Clostridium spp, Peptococcus spp, Peptostreptococcus spp, Prevotella spp*, or any other anaerobes in intra-abdominal abscess, peritonitis, empyema, pneumonia, aspiration pneumonia, lung abscess, diabetic foot ulcer, meningitis and brain abscess, bone and joint infections
- *H. pylori* eradication therapy, as part of a multi-drug regimen in peptic ulcer disease
- Amoebiasis
- Giardiasis
- Trichomoniasis

**Dosage and administration**

Metronidazole may be taken orally with or without food. In the hospital, metronidazole can be administered intravenously to treat serious infections. The liver is primarily responsible for eliminating metronidazole from the body, and doses may need to be reduced in patients with liver disease and abnormal liver function. *H. pylori*: 800-1500 mg orally daily for 14 days in combination with other drugs [139].

**Adverse effects**

Common adverse drug reactions associated with systemic metronidazole therapy include: nausea, diarrhoea, and/or metallic taste in the mouth. Intravenous administration is commonly associated with thrombophlebitis. Infrequent adverse effects include: hypersensitivity reactions
(rash, itch, flushing, fever), headache, dizziness, vomiting, glossitis, stomatitis, dark urine, and/or paraesthesia [140]. High doses and/or long-term systemic treatment with metronidazole is associated with the development of black hairy tongue, leucopenia, neutropenia, increased risk of peripheral neuropathy and/or CNS toxicity. Metronidazole is listed by the US National Toxicology Program (NTP) as reasonably anticipated to be a human carcinogen. It has been shown to cause cancer in experimental animals. Yet, metronidazole was shown to be safe in humans. It appears to have a fairly low potential for cancer risk and under most circumstances the benefits of treatment outweigh the risk. Metronidazole is listed as a possible carcinogen according to the WHO International Agency for Research on Cancer (IARC) [40].

**4.17.2.4 Ranitidine bismuth citrate**

**Generic name:** Ranitidine bismuth citrate  
**Brand name:** *Tritec*  
**Drug category** histamine receptor antagonists  
**Chemical formula** N-2-[5-Dimethylaminomethyl-2-furanyl methylthio]ethyl-N'-methyl-2-nitroethenediamine 2-hydroxy-1,2,3 propanetricarboxylate  

**Description**

Bismuth is a mild antibiotic. Citrate is a form of salt. Ranitidine bismuth citrate is used to decrease the amount of acid in the stomach and to treat *H. pylori*. Ranitidine bismuth citrate is most commonly used with antibiotics to treat this infection. Ranitidine bismuth citrate is a white to off-white amorphous powder. The approximate molecular formula is \([\text{C}_{13}\text{H}_{22}\text{N}_{4}\text{O}_{3}\text{S}]_{0.84}\ \text{Bi[ C}_{6}\text{H}_{5}\text{O}_{7}]_{0.94}\), and the approximate molecular weight is 651. It is readily soluble in water. Each TRITEC (ranitidine bismuth citrate) Tablet for oral administration contains 400 mg of ranitidine bismuth citrate, equivalent to approximately 162 mg of ranitidine
(base), 128 mg of trivalent bismuth, and 110 mg of citrate. Each aqueous film-coated tablet also contains the inactive ingredients FD&C Blue No. 2 Aluminum Lake, magnesium stearate, methylhydroxypropylcellulose, microcrystalline cellulose, Povidone K30, sodium carbonate (anhydrous), titanium dioxide, and triacetin [141].

**Indications**

TRITEC (ranitidine bismuth citrate) in combination with clarithromycin is indicated for the treatment of patients with an active duodenal ulcer associated with *H. pylori* infection. Most patients not eradicated of *H. pylori* following TRITEC (ranitidine bismuth citrate) plus clarithromycin treatment will have clarithromycin resistant *H. pylori* isolates. Therefore, for those patients who fail therapy, clarithromycin susceptibility testing should be done when possible. Patients with clarithromycin resistant *H. pylori* should not be treated with TRITEC (ranitidine bismuth citrate) plus clarithromycin or with regimens which include clarithromycin as the sole antimicrobial agent. The eradication of *H. pylori* has been demonstrated to reduce the risk of duodenal ulcer recurrence [142].

**Pharmacokinetics**

Following ingestion, ranitidine bismuth citrate dissociates in intragastric fluid, giving rise to ranitidine and soluble and insoluble forms of bismuth. Following a single oral 400-mg dose of TRITEC (ranitidine bismuth citrate) to healthy volunteers, mean (± SD) peak ranitidine plasma concentration of 455 (± 145.3) ng/mL occurred at 0.5 to 5 hours. The rate and extent of absorption of ranitidine derived from TRITEC (ranitidine bismuth citrate) increased proportionally with increasing doses up to 1,600 mg. Ranitidine plasma concentrations showed no evidence of accumulation during a 28-day dosing period [126]. Oral absorption of bismuth is variable. A mean (± SD) peak bismuth plasma concentration of 3.3
(± 2.0) ng/mL occurs at 15 to 60 minutes after a 400-mg dose. The rate and extent of absorption of bismuth from TRITEC (ranitidine bismuth citrate) do not increase with increasing doses up to 800 mg, but increase more than proportionally with increasing doses above 800 mg. The rate of absorption of bismuth derived from an 800-mg dose of TRITEC (ranitidine bismuth citrate) is decreased by 50%, and the extent of absorption is decreased by 25% when taken 30 minutes after a meal as compared to 30 minutes before a meal. The absorption of bismuth from an 800-mg dose of TRITEC (ranitidine bismuth citrate) increased when gastric pH exceeded 6. The increased pH resulted from the administration of an 800-mg dose of TRITEC (ranitidine bismuth citrate) given 3 hours previously. Mucosal penetration and absorption of bismuth from TRITEC (ranitidine bismuth citrate) are not affected by the degree of gastritis, the presence of H. pylori, or an active ulcer. Small amounts of bismuth accumulate in plasma during twice-daily dosing with TRITEC [143].

**Dosage and administration**

The recommended dosage of TRITEC (ranitidine bismuth citrate) is 400 mg b.i.d. for 4 weeks (28 days) in conjunction with clarithromycin 500 mg b.i.d. t.i.d. for the first 2 weeks (14 days). TRITEC (ranitidine bismuth citrate) and clarithromycin can be taken with or without food [144]. An alternative dosage regimen of TRITEC (ranitidine bismuth citrate) 400 mg b.i.d. for 4 weeks (28 days) in conjunction with clarithromycin 500 mg t.i.d. for the first 2 weeks (14 days) has been shown to be equally effective [144].

**Side effects**

Nausea, diarrhea, headache, or dizziness may occur at first as your body adjusts to the medication. If these effects persist or become bothersome, inform your doctor. Notify your doctor if you experience: unusual bleeding or bruising, pounding chest pain, rash, difficulty
sleeping, mental changes, extreme weakness, itching, breathing trouble, stomach pain. A temporary, harmless dark tongue or stool can occur [145].

4.18 Test drugs formulation

Herbal coded formulation of compound drugs with their synergistic action of herbal drugs design and calculated according to herbal pharmacopoeia, monographs of Unani medicine on scientific basis. Patients had been treated with herbal preparation of coded formulation.

Pylorex plus tablet:

Each 500mg tablet contains;

- *Curcuma longa* 150mg
- *Mellotus phillipenensis* 150mg
- *Glycyrrhiza glabra* 100mg
- *Zingiber officinale* 100mg

4.18.1 Manufacturing procedure

All the medicinal plant drugs that were designed for Pylorex plus were purchased from the Jodia market in Karachi. The details are as follows *Curcuma longa* rhizomes, *Mellotus phillipenesis* seeds, *Glycyrrhiza glabra* roots and rhizomes of *Zingiber officinale*. All the plant drugs were identified and authenticated by Prof. Dr. Usman Ghani Khan, Faculty of Eastern Medicine, Hamdard University, Karachi. All the drugs were cleaned thoroughly and grinded to make powder form. It is passed through the sieve to obtain the fine powder. Then binding agent are added and passed through a single punch machine to get fine tablets. All tablets are stored in a glass jar.

4.18.2 Pylorex plus formulation and selection criteria
Selection process involves the determination of action needed to address the multiplicity of symptoms presented as well as a basic understanding of the processes involved with \( H. \text{pylori} \) infection having researched this following the initial visit. It was determined that the following actions would be beneficial of coded herbal drug formulation.

\textit{Curcuma longa} contains many powerful anti-inflammatory and antioxidant compounds. It exerts a strong antibacterial effect against \( H. \text{pylori} \). Licorice extract produced a potent anti \( H. \text{pylori} \) effects. Liquorice commonly used in conventional medicine for both mouth ulcers and peptic ulcers and gastritis. Anti \( H. \text{pylori} \) activity of \textit{Mellotus philipenensis} have been evaluated in many studies especially against clarithromycin resistant (CR) and metronidazole resistant (MR) strains. It could be hopefully utilized for the development of new antimicrobial agents to prevent \( H. \text{pylori} \) related disorders. Ginger extract may decrease \( H. \text{pylori} \)-induced acute and chronic inflammatory process through the inhibition of a number of components of this pro-inflammatory signaling pathway.
### 4.19 Clinical trial protocol for *H. pylori* infection

<table>
<thead>
<tr>
<th>Patient Name</th>
<th>F/H Name</th>
<th>Age</th>
<th>Sex</th>
<th>Date</th>
<th>Patient CNIC</th>
<th>Contact No.</th>
<th>Patient ID</th>
</tr>
</thead>
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<tr>
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<td></td>
<td></td>
</tr>
<tr>
<td>Address</td>
<td>Investigator/Researcher</td>
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</table>

<table>
<thead>
<tr>
<th>Presenting Complaints</th>
<th>Past History</th>
<th>Previous Drug History</th>
<th>Family History</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

#### Associated Disorders

- Hypertension
- Diabetes Mellitus
- CVS disorders
- Obesity
- Surgical History

#### General Physical Examination

<table>
<thead>
<tr>
<th>Visit Date</th>
<th>Anaemia</th>
<th>Clubbing</th>
<th>Cyanosis</th>
<th>Oedema</th>
<th>Koilonychias</th>
<th>Jaundice</th>
</tr>
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<tbody>
<tr>
<td></td>
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</tr>
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</table>

<table>
<thead>
<tr>
<th>Vital signs</th>
<th>Blood pressure</th>
<th>Temperature</th>
<th>Pulse rate</th>
<th>Respiratory rate</th>
</tr>
</thead>
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<tr>
<td></td>
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</tr>
</tbody>
</table>

#### Signs and Symptoms

<table>
<thead>
<tr>
<th>Visit Date</th>
<th>Pain abdomen</th>
<th>Regurgitation</th>
<th>Heart burning</th>
<th>Indigestion/Flatus</th>
<th>Nausea/Vomiting</th>
<th>Belching</th>
</tr>
</thead>
<tbody>
<tr>
<td>1st week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2nd week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3rd week</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4th week</td>
<td></td>
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</tr>
</tbody>
</table>

#### Investigations

<table>
<thead>
<tr>
<th>Stool Antigen</th>
<th>Urea Breath</th>
<th>Rapid Ureas</th>
<th>Endoscopy</th>
<th>Culture/Histology</th>
<th>LFT’s</th>
<th>CBC</th>
</tr>
</thead>
<tbody>
<tr>
<td>Treatment</td>
<td>Control drug</td>
<td>Test drug</td>
<td>Improvement</td>
<td>Moderate Improvement</td>
<td>Slight Improvement</td>
<td>No Improvement</td>
</tr>
<tr>
<td>-----------</td>
<td>--------------</td>
<td>-----------</td>
<td>-------------</td>
<td>---------------------</td>
<td>-------------------</td>
<td>--------------</td>
</tr>
</tbody>
</table>

**Conclusion/Findings**

Major side effects observed

**Physician Name**

**Sign of Supervisor**

Patient Consent
CHAPTER -V

RESULT AND DISCUSSION
5. RESULTS AND DISCUSSION

Quadruple therapy consisting on bismuth compound has been documented as emerging therapy to eradicate *H. pylori* infection. Eradication rates by this therapy have been reported as equal to triple therapy based on clarithromycin. Recently, in a meta-analysis 5 randomized trials reported 79% and 85% eradication rates for clarithromycin triple therapy and bismuth based quadruple therapy showed 80% and 87% respectively. But this is disadvantageous and has been criticised due to its high tablet count with many side effects [146].

Moreover, unfortunately, triple therapy or bismuth based quadruple therapy produces eradication rates less than 85% which are decreasing further [146]. The most important factor for this failure is anti-*H. pylori* treatment is poor compliance and antibiotic resistance. New medicinal agents with good efficacy and less adverse effects are need of time in order to overcome this problem. Herbal medicine could be a choice to treat *H. pylori* infection and relieving the clinical sign and symptoms.

The study presented in this dissertation is a case control, multicenter, prospective randomized two arm parallel group clinical trial. The 176 patients suffering from *H. pylori* infection were randomized to the Pylorex plus and quadruple allopathic groups. Stool antigen test (HpSAg) was performed at baseline and after 4 weeks of treatment to access the eradication of *H. pylori*. The clinical assessment included the improvement in abdominal pain, regurgitation, heart burning, indigestion and flatulence, nausea, vomiting and belching. The data on clinical proforma was gathered and subjected to statistical analysis.

Likert scale was used to analyze the intensity of symptoms (scored as absent:0, mild:1, moderate:2, severe:3) such as abdominal pain, heart burning, regurgitation, indigestion and
flatulence, nausea, vomiting, belching at baseline (T0), after 2 week (T2) and after 4 weeks of treatment (T4). Median values and interquartile ranges (IQR) were recorded to represent the level of improvement. Routine examination of different investigations mentioned in clinical trial protocol especially urea breath test and stool antigen tests were done for assessing the improvement and other negative effects of medicines.

All this data was statistically analyzed by Chi–Square and the level of significance were applied. In order to validate the results alternative statistical analysis such as Exact Fisher Test was applied to confirm the efficacy of the treatment groups both in test and control group as Pylorex plus and Quadruple allopathic treatment respectively. The therapeutic evaluations of these medicines were conducted on 176 clinically and immunologically diagnosed cases of *H. pylori* infection at Shifa-ul-Mulk Memorial Hospital, for Eastern Medicine, Hamdard University Karachi, Matab Hakeem N. Salik, Rawalpindi and Bahawalpur Victoria Hospital Bahawalpur. All the patients were thoroughly examined and clinical history was taken and maintained on the proforma of case sheet enclosed herewith in the thesis. Both the drugs were evaluated on the basis of improvement in the clinical features and pathological investigations during the course of treatment at periodic intervals. These data was collected in the years April 2010-March 2012 which completed the clinical trial protocol. Consent of patient was taken at the first examination. The intent-to-treat population consisted of 176 patients enrolled: 86 were given coded herbal formulation Pylorex plus and 90 were prescribed Quadruple allopathic therapy. The analysis and evaluation on an intention to treat basis was included and only those participants taken who were willing to undergo treatment as well to attend all the follow up visits during the clinical trial. The primary outcome of this study was to eradicate the *H. pylori*. Secondary outcome was to treat the sign and symptoms of *H. pylori* infection.
In the collected data of 176 patients, male patients were 97 while 79 female patients were enrolled into the study. These 176 patients have been selected after the final selection from 210 patients. Out of remaining 34 patients, 15 patients did not agreed to participate in the clinical trial, 05 patients were dropped out due to poor response in follow up, 10 patients were excluded due to some serious side effects and remaining 04 patients were dropped out due to allergic reaction during the course of treatment.

After exclusion of drop-outs (changes in according to exclusion/inclusion criteria), the sample population with *H. pylori* infection comprised of 176 patients who had fulfilled the criteria at baseline. The patient’s gender, age, and baseline clinical features at the time of enrolment were recorded in both treatment arms. So overall, 176 patients were selected and 86 patients (48.86%) assigned to herbal coded formulation Pylorex plus and Quadruple therapy was prescribed to 90 patients (51.14%). During treatment clinical evaluation proforma was filled up which was designed on the basis of clinical evaluation and assessment of improvement in clinical signs and symptoms and record of the side effects encountered during the treatment.

### 5.1 Patient characteristics

Baseline Characteristics of the patients are given in Table 3. The mean ages and standard deviations of patients prescribed Pylorex plus as calculated were 28.14 ±9.43 and 27.82 ±9.01 years of males and females respectively. The mean age of patient prescribed Quadruple allopatic treatment as calculated was 27.95 ±9.80 and 29.49 ±10.02 years of males and females respectively as given in Table 4 and Graph 1.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Male</td>
<td>97 (55.11)</td>
</tr>
</tbody>
</table>
Female 79 (44.88)
Age (yr)
Mean ± SD 36±12
Range 15-45
Occupation
Government employee 48 (27.27)
Industrialist 52 (29.54)
Agriculturist 25 (14.2)
Miscellaneous 51 (28.97)
Economical status (PKR)
30,000-40,000 84 (47.72)
20,000-30,000 29 (16.47)
20,000-10,000 12 (6.81)
<10,000 51 (28.97)
Tobacco smokers 58 (32.95)
Alcohol 2 (1.13)

<table>
<thead>
<tr>
<th>Treatment groups</th>
<th>Sex</th>
<th>Mean</th>
<th>Number (n)</th>
<th>Standard Deviation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test drug (Pylorex plus)</td>
<td>Male</td>
<td>28.14</td>
<td>52</td>
<td>9.43</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>27.82</td>
<td>34</td>
<td>9.01</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>26.55</td>
<td>86</td>
<td>9.40</td>
</tr>
<tr>
<td>Control drug (Quadruple therapy)</td>
<td>Male</td>
<td>27.95</td>
<td>45</td>
<td>9.80</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>29.49</td>
<td>45</td>
<td>10.02</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>28.37</td>
<td>90</td>
<td>8.83</td>
</tr>
<tr>
<td>Total</td>
<td>Male</td>
<td>27.75</td>
<td>97</td>
<td>8.91</td>
</tr>
<tr>
<td></td>
<td>Female</td>
<td>28.30</td>
<td>79</td>
<td>8.71</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>27.46</td>
<td>176</td>
<td>8.56</td>
</tr>
</tbody>
</table>
Graph 1: Number of male and female patient prescribed test and control drugs

The age distribution we were done and all the patients were classified in different class interval ranging from 15 years to 45 years. 176 patients were classified into 3 class intervals accordingly, 15-24, 25-34 and 35-45 as shown in Table 5 and Graph 2. Between 15-24 years of age, the total numbers of patients were 78, between 25-34 years of age, the total numbers of patients were 52 and between 35-45 years of age, the total recorded patients were 45.

Table 5: Distribution of age

<table>
<thead>
<tr>
<th>Age groups</th>
<th>Treatment groups</th>
<th>Total (n)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test (n)</td>
<td>Control (n)</td>
</tr>
<tr>
<td>15 – 24Years</td>
<td>36</td>
<td>41</td>
</tr>
<tr>
<td>25 – 34 Years</td>
<td>26</td>
<td>27</td>
</tr>
<tr>
<td>35 – 45 Years</td>
<td>24</td>
<td>22</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>90</td>
</tr>
</tbody>
</table>
5.2 Treatment assignment and follow-up

One seventy six patients consented to participate in the study. Pretreatment clinical and laboratory parameters for the treatment groups were noted. The two treatment groups were comparable in efficacy results and side effects of the medicine administered. All subjects were clinically studied and completed the assigned therapy during the course of treatment.

In a trial, effect of Pylorex plus 500 mg 2 tablets after meal twice daily compared to Quadruple allopathic therapy was investigated on a total of 176 patients suffering from *H. pylori* infection for 15 days. Both preparations led to eradication of *H. pylori*.

Quadruple allopathic therapy that include Omeprazole 20mg b.i.d 15 minutes before meal, Amoxicillin 1g b.i.d, Metronodazole 500mg b.i.d after meal and TRITEC (ranitidine bismuth citrate) is 400mg t.i.d were prescribed to 90 patients.

5.3 Results

*H. pylori* eradication status

According to the statistical analysis *H. pylori* was eradicated in 51 patients (56.66%) out of 90 patients by the use of Quadruple allopathic therapy (Control drug) and in 53 patients...
(61.62%) out of 86 patients by the use of Pylorex plus (Test drug). Chi-Square Test was applied and p-value was calculated as 0.3031 which is greater than 0.05 (Table 7, Graph 3) indicating that Pylorex plus and Quadruple therapy are equally significant in *H. pylori* eradication.

**Table 6: *H. pylori* positive in total patients at baseline**

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Treatment groups</th>
<th>Total (n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test (Pylorex plus)</td>
<td>Control (Quadruple therapy)</td>
<td></td>
</tr>
<tr>
<td>H. pylori (Stool Antigen)</td>
<td>Positive</td>
<td>86</td>
<td>90</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>90</td>
<td>176</td>
</tr>
</tbody>
</table>

**Table 7: *H. pylori* eradication after treatment**

<table>
<thead>
<tr>
<th>After treatment</th>
<th>Treatment groups</th>
<th>Total (n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test (Pylorex plus)</td>
<td>Control (Quadruple therapy)</td>
<td></td>
</tr>
<tr>
<td>H. pylori eradication (Stool Antigen)</td>
<td>Negative</td>
<td>53 (61.62%)</td>
<td>51 (56.66%)</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>33 (38.37%)</td>
<td>39 (43.33%)</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>90</td>
<td>176</td>
</tr>
</tbody>
</table>
5.4 Urea Breath Test

This test was performed before the start of treatment in both test and control groups. In control group, a total of 90 patients were recorded as positive *H. pylori*, in which 23 patients were evaluated as positive by Urea Breath test before treatment. After treatment 14 patients were recorded negative test and 09 patients were recorded as positive Urea Breath test.

In test group, a total of 86 patients were recorded as positive *H. pylori*, in which 25 patients were evaluated as positive by Urea Breath test before treatment. After treatment 15 patients were recorded as having negative test and 10 patients were recorded as positive Urea Breath test. Overall 48 patients were evaluated by urea breath test both in test and control groups (Table 9, Graph 4).
Table 8: Urea Breath Test in total patients at baseline

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Treatment groups</th>
<th>Total (n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test (Pylorex plus)</td>
<td>Control (Quadruple therapy)</td>
<td></td>
</tr>
<tr>
<td>Urea Breath Test</td>
<td>Positive</td>
<td>25</td>
<td>23</td>
</tr>
<tr>
<td></td>
<td>Negative</td>
<td>00</td>
<td>00</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>25</td>
<td>23</td>
</tr>
</tbody>
</table>

Table 9: Urea Breath Test after treatment

<table>
<thead>
<tr>
<th>After treatment</th>
<th>Treatment groups</th>
<th>Total (n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test (Pylorex plus)</td>
<td>Control (Quadruple therapy)</td>
<td></td>
</tr>
<tr>
<td>Urea Breath Test</td>
<td>Negative</td>
<td>15</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>Positive</td>
<td>10</td>
<td>09</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>25</td>
<td>23</td>
</tr>
</tbody>
</table>
5.5 Improvement in *H. pylori* associated symptoms

All the clinical features were recorded at baseline and after the treatment and comparative analysis were done between test and control drugs to record the level of improvement by both the groups. *p* value < 0.05 was calculated by applying Chi-square test was considered as significant.

5.5.1 Abdominal pain

There was no difference between test and control groups at base line in abdominal pain. After treatment in test and control groups; test group (Pylorex plus) showed 85% improvement as compared to control (Quadruple allopathic therapy) 57% improvement. Significance test was applied and it was concluded that there was significant difference between these two drugs as *p*-value was calculated 0.0047 (*Table 11 and Graph 5*).
Table 10: Abdominal pain in total patients at baseline

<table>
<thead>
<tr>
<th>Baseline Abdominal pain</th>
<th>Treatment groups</th>
<th>Total (n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test (Pylorex plus)</td>
<td>Control (Quadruple therapy)</td>
<td></td>
</tr>
<tr>
<td>Yes</td>
<td>80</td>
<td>85</td>
<td>176</td>
</tr>
<tr>
<td>No</td>
<td>06</td>
<td>05</td>
<td>00</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>90</td>
<td>176</td>
</tr>
</tbody>
</table>

Table 11: Improvement in Abdominal pain after treatment

<table>
<thead>
<tr>
<th>After treatment Abdominal pain</th>
<th>Treatment Group</th>
<th>Total (n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test (Pylorex plus)</td>
<td>Control (Quadruple therapy)</td>
<td></td>
</tr>
<tr>
<td>Complete improvement</td>
<td>68 (85%)</td>
<td>57 (67.0%)</td>
<td>125</td>
</tr>
<tr>
<td>No improvement</td>
<td>12 (15%)</td>
<td>28 (32.9%)</td>
<td>40</td>
</tr>
<tr>
<td>Total</td>
<td>80</td>
<td>85</td>
<td>165</td>
</tr>
</tbody>
</table>
Graph 5: Improvement in Abdominal pain by use of Pylorex plus and Quadruple therapy

The p value calculated by the chi-square test is shown in these tables which indicate that overall a larger number of patients showed improvement in reducing the complaint of abdominal pain when treated with Pylorex plus as compared to improvement treated with Quadruple therapy.

5.5.2 Heart burning

Pylorex plus was prescribed to 62 patients suffering from heart burning. 52 patients showed complete improvement and 10 patients showed no improvement after the treatment with herbal drug Pylorex plus.

Quadruple allopathic therapy was prescribed to 70 patients with complaint of heart burning. After the treatment with quadruple allopathic therapy 65 out of 70 showed complete improvement and 5 patients showed no improvement.

Test group (Pylorex plus) showed 83.8% improvement and control (Quadruple allopathic therapy) showed 87% improvement. Significance test was applied and it was concluded that there was significant difference between these two drugs as p-value was calculated 0.137 as shown in Table 13 and Graph 6 which indicate that Quadruple therapy have good efficacy in
the improvement of heart burning.

Table 12: Heart burning in total patients at baseline

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Test (Pylorex plus)</th>
<th>Control (Quadruple therapy)</th>
<th>Total (n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart burning</td>
<td>yes</td>
<td>62</td>
<td>71</td>
<td>133</td>
</tr>
<tr>
<td></td>
<td>no</td>
<td>24</td>
<td>19</td>
<td>43</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>90</td>
<td>176</td>
<td>0.512</td>
</tr>
</tbody>
</table>

Table 13: Heart burning in total patients after treatment

<table>
<thead>
<tr>
<th>Complaint after treatment</th>
<th>Test (Pylorex plus)</th>
<th>Control (Quadruple allopathic therapy)</th>
<th>Total (n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart burning</td>
<td>improved</td>
<td>52 (83.8%)</td>
<td>65 (87.32%)</td>
<td>117</td>
</tr>
<tr>
<td></td>
<td>not improved</td>
<td>10 (16.12%)</td>
<td>06 (8.45%)</td>
<td>16</td>
</tr>
<tr>
<td>Total</td>
<td>62</td>
<td>71</td>
<td>133</td>
<td>0.137</td>
</tr>
</tbody>
</table>

Graph 6: Improvement in heart burning by Pylorex plus and Quadruple therapy
5.5.3 Regurgitation

Pylorex plus was prescribed to 47 patients with complaint of regurgitation. After the treatment, 45 patients showed complete improvement and 02 patients showed no improvement. Quadruple allopathic therapy was prescribed to 54 patients with complaint of regurgitation. After the treatment with quadruple allopathic therapy, 36 out of 54 showed complete improvement and 36 patients showed no improvement.

There was little difference between test and control group in regurgitation before treatment. After treatment, test group (Pylorex plus) has 95.7% improvement as compared to control (Quadruple therapy) 33.33% improvement. Significance test was applied and it was concluded that there was significant difference between these two drugs as p-value was calculated 0.000 as given in Table 15 and Graph 7.

Table 14: Regurgitation in total patients at baseline

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Treatment groups</th>
<th>Total (n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test (Pylorex plus)</td>
<td>Control (Quadruple therapy)</td>
<td></td>
</tr>
<tr>
<td>Regurgitation No</td>
<td>47</td>
<td>54</td>
<td>101</td>
</tr>
<tr>
<td>Yes</td>
<td>39</td>
<td>36</td>
<td>75</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>90</td>
<td>176</td>
</tr>
</tbody>
</table>

Table 15: Regurgitation in total patients after treatment

<table>
<thead>
<tr>
<th>After treatment</th>
<th>Treatment groups</th>
<th>Total (n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Regurgitation</td>
<td>Test (Pylorex plus)</td>
<td>Control (Quadruple therapy)</td>
<td></td>
</tr>
<tr>
<td>Improved</td>
<td>45 (95.7%)</td>
<td>36 (66.66%)</td>
<td>81</td>
</tr>
<tr>
<td>Not Improved</td>
<td>02 (4.25%)</td>
<td>18 (33.33%)</td>
<td>20</td>
</tr>
</tbody>
</table>
95.7% of patients prescribed Pylorex plus had complete improvement and 33.33% of patients prescribed Quadruple therapy had complete improvement.

<table>
<thead>
<tr>
<th></th>
<th>Total</th>
<th>47</th>
<th>54</th>
<th>101</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Regurgitation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improved</td>
<td>45</td>
<td>36</td>
<td>18</td>
<td></td>
</tr>
<tr>
<td>Not Improved</td>
<td>2</td>
<td>18</td>
<td>36</td>
<td>45</td>
</tr>
</tbody>
</table>

**Graph 7: Improvement in regurgitation by Pylorex plus and Quadruple therapy**

### 5.5.4 Indigestion and flatulence

Pylorex plus was prescribed to 67 patients with complaint of indigestion and flatulence. After the treatment with herbal drug Pylorex plus, 55 out of 67 showed complete improvement and 12 patients showed no improvement. Quadruple allopathic therapy was prescribed to 75 patients with complaint of indigestion and flatulence. After the treatment with quadruple allopathic therapy, 52 patients showed complete improvement and 23 patients showed no improvement.

Pylorex plus has 82.08% improvement as compared to Quadruple allopathic therapy which showed 69.33% improvement. Significance test was applied and it was concluded that there was significant difference between these two drugs as p-value was calculated 0.0580 (Table 17 and Graph 8).
Table 16: Indigestion and flatulence in total patients at baseline

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Treatment Group</th>
<th>Total (n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test (Pylorex plus)</td>
<td>Control (Quadruple therapy)</td>
<td></td>
</tr>
<tr>
<td>Indigestion/flatulence</td>
<td>Yes</td>
<td>67</td>
<td>75</td>
</tr>
<tr>
<td></td>
<td>No</td>
<td>19</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>86</td>
<td>90</td>
</tr>
</tbody>
</table>

Table 17: Indigestion and flatulence in total patients after treatment

<table>
<thead>
<tr>
<th>After treatment</th>
<th>Treatment Group</th>
<th>Total (n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test (Pylorex plus)</td>
<td>Control (Quadruple therapy)</td>
<td></td>
</tr>
<tr>
<td>Indigestion/flatulence</td>
<td>Improved</td>
<td>55 (82.08%)</td>
<td>52 (69.33%)</td>
</tr>
<tr>
<td></td>
<td>Not Improved</td>
<td>12 (17.91%)</td>
<td>23 (30.66%)</td>
</tr>
<tr>
<td>Total</td>
<td></td>
<td>67</td>
<td>75</td>
</tr>
</tbody>
</table>

82.08% of patients prescribed Pylorex plus were completely improved and 30.66% of patients prescribed Quadruple therapy showed complete improvement.
Graph 8: Improvement in Indigestion and flatulence by Pylorex plus and quadruple therapy

5.5.5 Nausea and vomiting

Pylorex plus was prescribed to 33 patients with complaint of nausea and vomiting. After the treatment with herbal drug Pylorex plus, all 33 patients showed complete improvement. Quadruple allopathic therapy was prescribed to 39 patients with complaint of nausea and vomiting. After the treatment with quadruple allopathic therapy, 33 out of 39 showed complete improvement and 6 patients showed no improvement.

There was little difference between test and control groups in nausea and vomiting before treatment. After treatment, test group (Pylorex plus) showed 100% improvement and control drug (Quadruple allopathic therapy) showed 84.61% improvement. Test of significance was applied and it was concluded that there was significant difference between these two drugs as p-value was calculated 0.020 (Table 19 and Graph 9).
Table 18: Nausea and vomiting in total patients at baseline

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Treatment groups</th>
<th>Total (n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test (Pylorex plus)</td>
<td>Control (Quadruple therapy)</td>
<td></td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>Present</td>
<td>33</td>
<td>39</td>
</tr>
<tr>
<td></td>
<td>Absent</td>
<td>53</td>
<td>51</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>86</td>
<td>90</td>
</tr>
</tbody>
</table>

Table 19: Nausea and vomiting in total patients after treatment

<table>
<thead>
<tr>
<th>After treatment</th>
<th>Treatment Group</th>
<th>Total (n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test (Pylorex plus)</td>
<td>Control (Quadruple therapy)</td>
<td></td>
</tr>
<tr>
<td>Nausea and vomiting</td>
<td>Improved</td>
<td>33 (100%)</td>
<td>33 (84.61%)</td>
</tr>
<tr>
<td></td>
<td>Not Improved</td>
<td>00</td>
<td>06 (15.38%)</td>
</tr>
<tr>
<td></td>
<td>Total</td>
<td>33</td>
<td>39</td>
</tr>
</tbody>
</table>

100% of patients were prescribed Pylorex plus showed complete improvement and 84.61% of patients were prescribed quadruple allopathic therapy showed complete improvement.
5.5.6 Belching

Pylorex plus was prescribed to 63 patients with complaint of belching. After the treatment with herbal drug Pylorex plus, 58 patients showed complete improvement while 05 patients showed no improvement. Quadruple allopathic therapy was prescribed to 66 patients with complaint of belching. After the treatment with quadruple allopathic therapy, 51 out of 66 showed complete improvement and 15 patients showed no improvement.

There was little difference between test and control groups in level of belching before treatment. After treatment in test and control group, test group (Pylorex plus) showed 92.06% improvement and control (Quadruple allopathic therapy) showed 77.27% improvement. Test of significance was applied and it was concluded that there was significant difference between these two drugs as p-value was calculated 0.008 (Table 21 and Graph 10).
Table 20: Belching in total patients at baseline

<table>
<thead>
<tr>
<th>Baseline</th>
<th>Treatment groups</th>
<th>Total (n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test (Pylorex plus)</td>
<td>Control (Quadruple therapy)</td>
<td></td>
</tr>
<tr>
<td>Belching</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Present</td>
<td>63</td>
<td>66</td>
<td>129</td>
</tr>
<tr>
<td>Absent</td>
<td>23</td>
<td>24</td>
<td>47</td>
</tr>
<tr>
<td>Total</td>
<td>86</td>
<td>90</td>
<td>176</td>
</tr>
</tbody>
</table>

Table 21: Belching in total patients after treatment

<table>
<thead>
<tr>
<th>After treatment</th>
<th>Treatment Group</th>
<th>Total (n)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Test (Pylorex plus)</td>
<td>Control (Quadruple therapy)</td>
<td></td>
</tr>
<tr>
<td>Belching</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Improved</td>
<td>58 (92.06%)</td>
<td>51 (77.27%)</td>
<td>109</td>
</tr>
<tr>
<td>Not Improved</td>
<td>05 (7.93%)</td>
<td>15 (22.72%)</td>
<td>20</td>
</tr>
<tr>
<td>Total</td>
<td>63</td>
<td>66</td>
<td>49</td>
</tr>
</tbody>
</table>
A comparative analysis was done in the improvement of \textit{H. pylori} infection associated sign and symptoms. It was noted that Pylorex plus tablet (Test drug) showed overall superior results in the improvement of many subjective sign and symptoms as compared to Quadruple therapy (Table 22). The results from this study have clearly revealed the evidence of efficacy in real terms.

Table 22: Overall comparative evaluation in improvement of sign/symptoms by Pylorex plus and Quadruple therapy

<table>
<thead>
<tr>
<th>Sing/ Symptoms</th>
<th>Treatment Groups</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pylorx plus (Herbal)</td>
<td>Quadruple therapy</td>
</tr>
<tr>
<td></td>
<td>Improved</td>
<td>Not Improved</td>
</tr>
</tbody>
</table>
| Abdominal pain | 68/80     | 12/80        | 85% | 57/85     | 28/85        | 67% | 0.005
| Heart burning  | 52/80     | 10/80        | 65% | 65/85     | 6/85         | 87% | 0.137
| Regurgitation  | 45/47     | 2/47         | 95.7% | 36/54    | 18/54        | 66.6% | 0.002
| Indigestion/ Flatulence | 55/67 | 12/67        | 82.08% | 52/75    | 23/75        | 69.3% | 0.058
| Nausea/ vomiting | 33/33 | 00/33        | 100% | 33/39    | 6/39         | 84.6% | 0.0209
| Belching       | 21/23 | 2/23         | 57.6% | 15/26    | 11/26        | 57.6% | 0.008
5.6 Intensity of symptoms

There was a significant improvement in *H. pylori* associated symptoms in test group as compared to control group when observed between these two treated groups at the end of therapy. We recorded the intensity of symptoms as absent: 0, mild: 1, moderate: 2 and sever: 3 at baseline (T0), 2\textsuperscript{nd} week of treatment (T2) and after 4 weeks (T4) of treatment through median values, interquartile ranges (IQR) and Wilcoxon signed-rank test was applied to calculate differences in median values.

In test group a statistically significant decrease in the overall dyspeptic symptom score was observed from baseline (T0: median 8, IQR 6-10) to 2\textsuperscript{nd} week (T2: median 3, IQR 2-6) and one month after treatment (T4: median 3.5, IQR 3-7). Quadruple therapy also exhibited a statistically significant decrease in the overall dyspeptic symptom score from baseline (T0: median 9, IQR 7-11) to 2\textsuperscript{nd} week (T2: median 4, IQR 3-5) and one month after treatment (T4: median 6, IQR 3-7). In non *H. pylori* eradicated patients a marked symptomatic improvement was observed in test group in overall symptom score from baseline (T0: median 9, IQR 5-12) to one month after treatment (T4: median 4, IQR 2-6) as compared to quadruple therapy (T0: median 9, IQR 5-13) to one month after treatment (T4: median 8, IQR 5-10).

5.6.1 Improvement in symptoms with Pylorex plus

There was a statistically significant decrease in the overall dyspeptic symptom score from baseline (T0: median 8, IQR 6-10) to 2\textsuperscript{nd} week (T2: median 3, IQR 2-6) and one month after treatment (T4: median 3.5, IQR 3-7) as given in Table 23 and Graph11.
Table 23: Overall improvement in severity of symptoms in Test group by Wilcoxon Signed Rank Test

Overall severity of symptoms

<table>
<thead>
<tr>
<th></th>
<th>Baseline (T0)</th>
<th>End of treatment (T2)</th>
<th>After 1 moth of treatment (T4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Median IQR</td>
<td>8 6-10</td>
<td>3 2-6</td>
<td>3.5 3-7</td>
</tr>
<tr>
<td>p value</td>
<td>0.004</td>
<td>0.004</td>
<td>0.003</td>
</tr>
</tbody>
</table>

Graph 11: Overall Improvement in intensity of symptoms

Abdominal pain (T0: median 2.5, range 2-3; T2: median 1, range 1-2; T4: median 1, range 1-2), heart burning (T0: 2, range 1-3; T2: median 1, range 1-2; T4: median 0.5, range 1-2), regurgitation (T0: 2, range 2-3; T2: median 1, range 1-2; T4: median 1, range 1-2), indigestion and flatulence (T0: 2.5, range 2-3; T2: median 1, range 1-2; T4: median 0.5, range 1-3),
nausea/vomiting (T0: median 2.5, range 2-3; T2: median 1.5, range 1-2; T4: median 1, range 0-1) and belching (T0: median 2, range 2-3; T2: median 1, range 1-2; T4: median 1.5, range 1-2) all showed statistically significant improvement after treatment with non-antibiotic quadruple regimen. All symptom scores were showed in Table 24 and Graph12.

Table 24: Improvement in Intensity of symptoms with Pylorex plus tablet by Wilcoxon Signed Rank Test

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Baseline (T0)</th>
<th>After 2 weeks (T2)</th>
<th>1 month after treatment (T4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>IQR</td>
<td>Median</td>
</tr>
<tr>
<td><strong>Abdominal pain</strong></td>
<td>2.5</td>
<td>2-3</td>
<td>1</td>
</tr>
<tr>
<td><strong>Heart burning</strong></td>
<td>2</td>
<td>1-3</td>
<td>1</td>
</tr>
<tr>
<td><strong>Regurgitation</strong></td>
<td>2</td>
<td>1-3</td>
<td>1</td>
</tr>
<tr>
<td><strong>Indigestion/ Flatulence</strong></td>
<td>2.5</td>
<td>2-3</td>
<td>1</td>
</tr>
<tr>
<td><strong>Nausea/ vomiting</strong></td>
<td>2.5</td>
<td>2-3</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Belching</strong></td>
<td>2</td>
<td>2-3</td>
<td>1</td>
</tr>
</tbody>
</table>
Graph 12: Intensity of abdominal pain, heart burning, regurgitation, indigestion/flatulence, nausea/vomiting and belching respectively, green arrow showing the median value.

The efficacy of herbal formulation is a characteristic of a complex mixture of chemical compounds present in different herbs used as multiple dosage form design. The clinical trial in case of Test drug, therefore, has been designed in a manner that reflects the characteristic bioactivity as used in ethnopharmacology.

5.6.2 Improvement profile with Quadruple therapy

Quadruple therapy also exhibited a statistically significant decrease in the overall dyspeptic symptom score from baseline (T0: median 9, IQR 7-11) to 2\textsuperscript{nd} week (T2: median 4, IQR 3-5) and one month after treatment (T4: median 6, IQR 3-7) as shown in Table 25 and Graph13.
Table 25: Overall severity of symptoms in control group by Wilcoxon Signed Rank Test

Overall severity of symptoms

<table>
<thead>
<tr>
<th></th>
<th>Baseline (T0)</th>
<th>End of treatment (T2)</th>
<th>After 1 month of treatment (T4)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall severity</td>
<td>Median</td>
<td>IQR</td>
<td>p value</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>7-11</td>
<td>0.003</td>
</tr>
<tr>
<td></td>
<td>4</td>
<td>3-5</td>
<td>0.01</td>
</tr>
<tr>
<td></td>
<td>6</td>
<td>3-7</td>
<td></td>
</tr>
</tbody>
</table>

Graph 13: Overall Improvement in intensity of symptoms

Abdominal pain (T0: median 2.5, range 2-3; T2: median 1.5, range 1-2; T4: median 2, range 1-2), heart burning (T0: median 2, range 2-3; T2: median 1, range 1-3; T4: median 1.5, range 1-3), regurgitation (T0: median 2.5, range 1-3; T2: median 1.5, range 1-2; T4: median 1, range 1-3), indigestion and flatulence (T0: 2.5, range 2-3; T2: median 1.5, range 1-2; T4: median 2, range 1-3), nausea/vomiting (T0: median 2, range 2-3; T2: median 1, range 1-3; T4: median 1.5, range 1-3) and belching (T0: median 2, range 2-3; T2: median 1, range 1-2; T4: median 1.5, range 1-3) all showed statistically significant improvement after treatment with quadruple
regimen. All symptom scores were showed in Table 26 and Graph 14.

Table 26: Improvement in Intensity of symptoms with Quadruple therapy

<table>
<thead>
<tr>
<th>Symptoms</th>
<th>Baseline (T0)</th>
<th>After 2 weeks (T2)</th>
<th>1 month after treatment (T4)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Median</td>
<td>IQR</td>
<td>Median</td>
</tr>
<tr>
<td><strong>Abdominal pain</strong></td>
<td>2.5</td>
<td>2-3</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Heart burning</strong></td>
<td>2</td>
<td>2-3</td>
<td>1</td>
</tr>
<tr>
<td><strong>Regurgitation</strong></td>
<td>2.5</td>
<td>1-3</td>
<td>1</td>
</tr>
<tr>
<td><strong>Indigestion/Flatulence</strong></td>
<td>2.5</td>
<td>2-3</td>
<td>1.5</td>
</tr>
<tr>
<td><strong>Nausea/vomiting</strong></td>
<td>2</td>
<td>1-3</td>
<td>1</td>
</tr>
<tr>
<td><strong>Belching</strong></td>
<td>2</td>
<td>2-3</td>
<td>1</td>
</tr>
</tbody>
</table>
Graph 14: Intensity of abdominal pain, heart burning, regurgitation, indigestion/flatulence, nausea/vomiting and belching respectively, green arrow showing the median values

5.6.3 Comparative analysis of intensity of symptoms between treatment groups

A comparative analysis was done in the level of intensity of symptoms between two treated groups i.e. test and control groups before and after the treatment. Wilcoxon signed rank test was applied to see the statistical difference after calculating the median values and interquartile ranges. It was concluded from this statistical analysis that Pylorex plus (Test) possesses greater value to lower down the intensity of symptoms as compared to Quadruple therapy (Control) as shown in Table 27 and Graph 15.
Table 27: Comparison in intensity of symptoms between two treatment groups by Wilcoxon Signed Rank Test

<table>
<thead>
<tr>
<th></th>
<th>Pylorex plus</th>
<th></th>
<th>Quadruple therapy</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Before</td>
<td>After 4 weeks of</td>
<td>Before</td>
<td>After 4 weeks of</td>
</tr>
<tr>
<td></td>
<td>treatment</td>
<td>treatment</td>
<td>treatment</td>
<td>treatment</td>
</tr>
<tr>
<td>Median</td>
<td>8</td>
<td>6-10</td>
<td>3.5</td>
<td>2-5</td>
</tr>
<tr>
<td>IQR</td>
<td>6</td>
<td>2</td>
<td>5</td>
<td>0.003</td>
</tr>
<tr>
<td>p value</td>
<td></td>
<td></td>
<td></td>
<td>0.01</td>
</tr>
</tbody>
</table>

Graph 15: Comparative analysis in intensity of symptoms between two treatment groups

Profile of *H. pylori* non-eradicated patients

*H. pylori* was not eradicated in 33 (38.37%) patients in test group while in 39 (43.33%) patients in control group after the completion of treatment but marked symptomatic improvement was observed in test group in overall symptom score from baseline (T0: median 9, IQR 5-12) to one month after treatment (T4: median 4, IQR 2-6) as compared to quadruple therapy (T0:
median 9, IQR 5-13) to one month after treatment (T4: median 8, IQR 5-10) as shown in Table 28 and Graph 16.

Table 28: Intensity of symptoms in *H. pylori* non-eradicated patients by Wilcoxon Signed Rank Test

<table>
<thead>
<tr>
<th></th>
<th>Pylorex plus</th>
<th>Quadruple therapy</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Before treatment</strong></td>
<td>Median 9, IQR 5-12</td>
<td>Median 9, IQR 5-13</td>
</tr>
<tr>
<td><strong>After 4 weeks of treatment</strong></td>
<td>Median 4, IQR 2-6</td>
<td>Median 8, IQR 5-10</td>
</tr>
<tr>
<td><strong>p value</strong></td>
<td>0.003</td>
<td>0.2</td>
</tr>
</tbody>
</table>

Graph 16: Comparative analysis in intensity of symptoms in non *H. pylori* between two treatment groups
Thompson and Ernst, 2002 had reported that herbal medicines have proved to be very safe and effective and now essential for the development of new medicinal agents to cure gastrointestinal disorders and many other ailments [147]. Therefore, herbal medicines having anti-\(H.\) pylori activities have been reported from India, Pakistan, Turkey, Japan, Taiwan and other different areas of the world. In many previous reports documented by Bae et al., 1998; Yesilada et al., 1999; Shin et al., 2004; Wang and Huang, 2005 have revealed promising antibacterial activities against \(H.\) pylori [148, 149, 150, 151]. There is a great need for proper screening documentations on these herbal medicines in South-Asian countries like India, Pakistan and Bangladesh where herbal system of medicine is widely used in preventive and curative purposes. Faisal et al., conducted a screening study in which anti-\(H.\) pylori activities of 50 commonly used traditional medicinal herbs from Pakistan were evaluated through aqueous-ethanol extract and minimum bactericidal concentrations were determined. These herbs are commonly prescribed in South-Asian countries for the treatment of gastrointestinal disorders. Out of 50 medicinal plants, Mallotus philipinesis (Lam) Muell, Curcuma amada Roxb., Myristica fragrans Houtt., and Psoralea corylifolia L. exhibited very potent anti-\(H.\) pylori activity. Mallotus philipinesis showed highest bactericidal activity arresting the growth of \(H.\) pylori at the concentration of 15.6–31.2g/ml [152].

Genetic diversity has been reported in the \(H.\) pylori population worldwide with inter-stain variations [155]. Faisal et al measured MBC values of the four most active extract of medicinal plants in three Japanese \(H.\) pylori strains to investigate whether these DNA samples can affect the bactericidal activity. MBC values between Pakistani and Japanese strains remains same which further strengthen the hypothesis that these medicinal plants can be prescribed in different geographically strains against \(H.\) pylori [152]. In clinical trials for eradication of \(H.\) pylori by
utilization of these medicinal plants is controversial but it is now confirmed that they may repress the pathogenicity of *H. pylori* by different mechanisms. Suppress IL-8 expression and NF-$kB$ activation has been noted by curcumin and capsaicin which are marker compounds isolated from medicinal plants in different culture experiments [156].

Mario *et al.*, 2007 recently reported in a clinical trial that curcumin-based one week triple therapy have marked results in the improvement of dyspeptic symptoms and reduction of gastric inflammatory response but was not so successful to eradicate *H. pylori*. It is hypothesized in this study that activation of the vanilloid receptor type 1 (TRPV1) by curcumin exerts beneficial effects, but still it is needed to evaluate the exact mechanisms of these constituents obtained from medicinal plants [157].

Anti-*H. pylori* activity of *Plumbago zeylanica* has been reported by Wang and Huang in 2005, which is also used for the treatment of intestinal parasites in Taiwanese traditional remedies. This correlation between anti-*H. pylori* activity and anthelmintic medicinal plants could be a trail for future screening of traditional medicines in search for novel compounds against *H. pylori* [150].

Curcuma species are widely known for their broad range of pharmacological activities. Usmanghani *et al.*, 1997 have documented that three varieties of Curcuma species namely *Curcuma amada* Roxb. (CAR), *Curcuma caesia* Roxb. (CCR), and *Curcuma longa* L. (CLL) are available in Pakistan. CAR and CLL are commonly used as a spice in daily life while CCR is mainly employed for medicinal purposes especially as an alternate of turmeric or CLL [138]. Faisal *et al.*, reported that among these three species, CAR (31.2–62.5g/ml) and CLL (62.5g/ml) showed strong inhibition on *H. pylori* growth in all strains while CCR (250g/ml) exhibited weak bactericidal activity in contrast with the other two [152].
Mahady et al., (2002) documented earlier that *Curcuma longa* L. and its major polyphenolic chemical constituent, curcumin has potent Anti-*H. pylori* activity whereas; Siddaraju and Dharmesh (2007) recently reported the anti-*H. pylori* activity of *Curcuma amada* Roxb [153, 154]. The MBC value of curcumin revealed from Faisal et al., study is ranged from 25.0 to 50.0g/ml which is also comparable with amoxicillin.

It has been reported in previous study that extract of ginger rhizomes inhibited the growth of 19 strains of *H. pylori* in vitro with a minimal inhibitory concentration range of 0.78to12.5 µg/mL, with significant activity against the cagA + positive strains. These data suggest that specific ginger extracts containing the gingerols 6-10 may be used for the treatment of *H. pylori* infection. Propolis and *Zingiber officinale* have been shown to be specifically targeted against *H. pylori* strains, to possess anti-inflammatory, antioxidant and anti-tumor activity and to be used in traditional medicine for the treatment of gastrointestinal ailments [158, 159].

Considering that these traditional products could potentially serve as novel therapeutic agents and taking the advantage of these reports, a formulation has been designed based on above literature citation. The coded herbal formulation Pylorex plus contains total four medicinal plants; *Curcuma longa, Mellotus phillipenensis, Zingiber officinale* and *Glycyrrhiza glabra* for the treatment of *H. pylori* infection.

This clinical trial was conducted between two treatment groups to validate the effectiveness and safety of. Pylorex plus as test drug and Quadruple allopathic therapy as control drug was prescribed for the treatment of *H. pylori* infection. Study was under taken as observational paradigms in which objectives have been defined as comparative evaluate herbal and allopathic medicine so as to assess their efficacy in *H. pylori* infection.

The object of this study was to compare herbal medicine Pylorex plus and Quadruple
allopathic medicine and to see whether these may represent a platform for the development of novel therapeutics. It was observed that there is a marked improvement in overall subjective signs and symptoms when treated with Pylorex plus as compared to quadruple therapy. There was a noticeable improvement in abdominal pain, regurgitation, nausea/vomiting and in belching which are the most common symptoms of active *H. pylori* infection. This may be the most striking findings of our study. We may hypothesized that this effect is due to the presence of curcumin in *Curcuma longa* which is major active ingredient of Pylorex plus. Curcumin activates vanilloid receptor type I (TRPV I) in the gastrointestinal tract and enteric nervous system which maintain the mucosal integrity against many bacterial agents, viruses, activated gastric enzymes and other aggressive compounds. It also prevents gastric epithelial cell damage. Thus it depresses the process of inflammation in gastric mucosal cell. The glycyrrhizin has also an anti-inflammatory action, it inhibits the production of PGE2 and increases the production of stomach mucus, the lifetimes of the epithelial cells of the stomach and inhibits the secretion of pepsinogen. The glycyrrhetic acid partially blocks the degradation of adrenal hormones, in particular the cortisol. Therefore, it prolongs their biological effects in humans. In vitro, the hydro alcoholic extract of Liquorice inhibits *H. pylori* strains with minimum inhibitory concentration (MIC) of 50 to 400 mg/ml [159].

The reduction in symptoms may also be due to the reduction in the level of serum pepsinogen and gastrin enzyme which are stimulant of inflammatory process in the stomach. So it can be assumed that Pylorex plus decreases the intensity of *H. pylori* related gastrointestinal inflammation in the stomach mucosa as well as low-down the level of serum pepsinogen and gastrin.

From the above discussion it is clearly evident that both therapies led to the reduction in
the *H. pylori* related gastrointestinal symptoms and it is noted that Pylorex plus possesses high level of improvement in the symptoms of *H. pylori* infection as compared to quadruple therapy. In some previous reports, it has been documented that Quadruple as well as triple therapies produces equal eradication rates as primary therapy for *H. pylori* infection. Patient compliance and side effects were also similar with both therapies.

In a previous report by Gomollon *et al.*, from Spain published in 2000, 48 patients were prescribed bismuth based quadruple therapy and 49 patients were given clarithromycin triple therapy in a randomized prospective clinical trial. 68.7 % of patients were cured receiving bismuth quadruple therapy and 81.6 % of patients were cured with clarithromycin triple therapy [160].

Katelaris *et al.*, conducted a multicenter study. in New Zealand and Australia in which 110 patients were randomized to conventional bismuth quadruple therapy and 104 patients to clarithromycin triple therapy. Higher metronidazole resistance was recorded in selected patients compared with clarithromycin resistance in both treatment groups. Eradication rate observed by bismuth quadruple therapy was 82% whereas clarithromycin triple therapy eradication rate was 78 % [161]. In United States and Canada a multi-center study was conducted by Laine *et al.*, in which 138 patients were randomized to receive bismuth quadruple therapy and 137 patients to receive clarithromycin triple therapy. *H. pylori* cure was documented with a 13 C-UBT after 29 and 57 days of treatment completion. The populations studied showed significantly greater resistance to metronidazole than clarithromycin. A total of 87.7 % of patients receiving bismuth quadruple therapy and 83.2 % of patients receiving clarithromycin triple therapy achieved successful *H. pylori* eradication [162]. In a study from Spain, Calvet *et al.*, randomized 168 patients to bismuth quadruple therapy and 171 patients to clarithromycin triple therapy.
Eradication was achieved in 83% of the bismuth quadruple therapy group and 77% of the clarithromycin triple therapy group [163].

Uygun et al., conducted a single-center study in which 120 patients received bismuth quadruple therapy and 120 patients received clarithromycin triple therapy. Study participants had non-ulcer dyspepsia and H. pylori infection documented by both UBT and histology. Post-treatment H. pylori status was determined with a UBT 6 weeks after the completion of treatment. The eradication rate was 70.0% with bismuth quadruple therapy and 57.5% with clarithromycin triple therapy. This study was carried out in Turkey where previous studies have documented high clarithromycin resistance rates and low eradication rates with clarithromycin-based clarithromycin triple therapy [164].

Pai et al., enrolled 33 and 35 patients with quadruple and triple therapies in a multicenter Indian study. H. pylori infection was successfully eradicated in 73 and 83% of the quadruple and clarithromycin triple therapy groups respectively [165]. Mantzaris et al., conducted a prospective, investigator blinded, single-center study involving patients with confirmed active duodenal ulceration by endoscopy and H. pylori infection by RUT and histology. The study included 71 patients in the bismuth quadruple therapy group and 78 patients in the clarithromycin triple therapy group. A total of 82 and 86% of patients in the quadruple and triple groups, respectively, were cured of H. pylori infection. This study was carried out in Greece, a country noted to have very high rates of metronidazole resistance [166].

In a study from Korea, Jang et al., bismuth quadruple therapy was randomized in 74 patients and clarithromycin triple therapy was prescribed to 75 patients. Eradication rates were 71.6 and 78.7% for the quadruple and clarithromycin triple therapy groups, respectively. Twelve patients receiving bismuth quadruple therapy and six patients in the clarithromycin triple therapy
group either did not complete therapy or were lost to follow-up [167].

From these studies it is clearly evident that primary triple and quadruple therapies produces equal eradication rates. Moreover, side effects and patients compliance rates are also similar by both treatment therapies. But unfortunately, both regimens yielded eradication rates below 80%. It is critically valuable to consider and address issues that might reduce antimicrobial resistance and enhance compliance rate.

Antibiotic resistance is a major cause of treatment failure [168]. The prevalence of antimicrobial resistance in *H. pylori* shows regional variation both within and between countries. Alternative antibiotics based on local resistance rates may improve eradication rates. Clarithromycin resistance has a greater effect on treatment efficacy than nitroimidazole resistance. The widespread and sometimes indiscriminate use of antibiotics in developing countries has resulted in a higher prevalence of resistance than in industrialized countries [169]. Clarithromycin resistance rates in the USA have a prevalence of 10–12.5% [170]. In Canada, clarithromycin resistance is estimated to be less than 4% [171]. In Europe, there is a significant difference between clarithromycin resistance rates in Northern, Eastern, and Southern Europe with resistance rates of 4.2%, 9.3%, and 18%, respectively [172]. The prevalence of secondary clarithromycin resistance, i.e. after failure of a treatment including this drug, is extremely high, up to 60%. Resistance to metronidazole is much more common than resistance to macrolides. In developed countries about 35% of *H. pylori* strains are resistant to metronidazole, whereas in developing countries the resistance rates are even higher [173]. The prevalence of amoxicillin resistance is low (< 1%). In areas where penicillin is available without prescription, it may be higher. Tetracycline resistance is estimated to be less than 1%. Fluoroquinolones are being increasingly prescribed in recent years and thus has led to increasing resistance rates. Patients
with failed *H. pylori* eradication had a higher chance of harboring multi-resistant *H. pylori* than untreated patients [174].

Hundreds of medicinal herbs are used in traditional system of medicine in all over the world for the treatment of bacterial infections. In vitro screening evaluation has been documented but clinical trials are lacking to confirm the efficacy of such herbal medicines.

These natural resources are usually safer than synthetic antibiotics and many physicians and patients prefer to use herbal medicines. Thus healthcare professionals should be aware of the available evidence for herbal antibiotics in the region. In a recent study, anti-*H. pylori* activity of commonly available Unani medicine was evaluated in Pakistan that are mostly used in GIT disorders to evaluate the natural source for active components against *H. pylori*. [81]. However, the results of clinical studies are variable.

5.7 Drug compliance and cost effectiveness

Compliance of the treatment and cost effectiveness of the both therapies used for the treatment of *H. pylori* infection was also analyzed during the course of treatment.

Table 29: Drug compliance and cost effectiveness comparison

<table>
<thead>
<tr>
<th>Remarks of the Patients</th>
<th>Treatment Group</th>
<th>Total</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pylorex plus</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>Quadruple therapy</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Drug Compliance</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Bad</strong></td>
<td>7</td>
<td>22</td>
<td>29</td>
</tr>
<tr>
<td><strong>Good</strong></td>
<td>79</td>
<td>68</td>
<td>147</td>
</tr>
<tr>
<td><strong>Cost Effectiveness</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Costly</strong></td>
<td>3</td>
<td>75</td>
<td>78</td>
</tr>
<tr>
<td><strong>Cost effective</strong></td>
<td>83</td>
<td>15</td>
<td>99</td>
</tr>
</tbody>
</table>

| 0.0079                  | 0.0001          |
The analysis through chi-square test revealed marked difference between two treated groups in term of cost effectiveness and drug compliance ($p < 0.05$). The comments about drug compliance and cost effectiveness were obtained at the end of treatment. The purpose is to determine whether the information obtained from the patients have any significant hearing and difference between the treatment groups.

5.8 Adverse effects profile

The majority of adverse events were assessed as mild in severity and self limiting in nature. But 06 patients were drop out due to some severe side effects and 04 patients due to allergic reaction in control group. Adverse events categorized by the clinical investigator as possibly drug related in patients administered quadruple allopathic therapy that showed diarrhea, (03 patients), headache (02 patients), anorexia (01 patient) and allergic reaction (04 patients) were the most common drug related events among control recipients. Whereas in test group, 04 patients showed some side effects such as headache (02 patients) and irritability (02 patients) and dropped out during the course of treatment. No life threatening side effects recorded in any group. It is because of the fact that plant drug selected for the treatment of *H. pylori* infection.
does not contain any chemical agent that may trigger the adverse drug reaction response. This can be explained further that chemical components of the plant drugs altogether are low in the frequency of occurrence and even administered together in synergistic fashion exhibit pronounced type of effective response for curative action.

5.9 Conclusion

The findings from this randomized clinical trial revealed that there was no statistically significant difference when comparing the effectiveness of herbal medicine Pylorex plus (Test) to quadruple allopathic therapy (Control) for the treatment of *H. pylori* infection. Furthermore, it is clearly evident that Pylorex plus possesses a therapeutic value in the improvement of *H. pylori* associated symptoms as compared to Quadruple allopathic therapy.

Chi-square test and Wlicoxone signed rank test were used to analyze the statistical differences between both therapies. From the statistical results obtained out of clinical response it was concluded that Pylorex plus is effective for the treatment of *H. pylori* infection and its associated symptoms, the effect being confirmed by physicians and patients alike.

There was no untoward clinical or pathological manifestation associated with the use of Pylorex plus and this has found good acceptability by all treated patients. The principal objective of the study was to compare Pylorex plus as compared to Quadruple allopathic therapy to determine whether these may represent a platform for the development of novel therapeutic. This is an exercise of applying modern techniques and clinical design to product that have been in use for centuries.

The results from this research study have clearly revealed the evidence of efficacy of test drug Pylorex plus for the eradication of *H. pylori* as well as marked improvement in its associated symptoms as compared to Quadruple allopathic therapy. However, further clinical
trials on larger scale and studies pertaining to mechanism of Pylorex plus are required before prescribing it as an alternate eradication therapy against *H. pylori*. In summary, this study outlines an approach to the scientific and clinical validation of alternate traditional and conventional medicines, so in its ultimate dictate; this is worthwhile exercise, since it leads to new class of therapeutics.
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1341–6; discussion 1346–7.


officinale Roscoe) and the gingerols inhibit the growth of CagA+ strains of H. pylori. *Anticancer Res.*, **23**:3699–3702


